ANIMAL BEHAVIOUR

Formerly The British Journal of Animal Behaviour

VOLUME VIII 1960

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EFFECTS OF RESTRAINT AND ISOLATION ON YELPING IN PUPPIES*

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Scott & Marston (1950) report that young puppies below the age of twelve weeks are often observed to give a rapid series of whines or yelps at rates rising to over 100 per minute. This behaviour appears to be associated with various situations of obvious discomfort: hunger, cold, confinement, isolation, etc. It may be called "distress vocalization" and can be classified as et-epimiletic or care-soliciting behaviour. Yelping can be easily quantified by counting the number of discrete sounds. The purpose of this study was to explore the effects of some environmental factors which affect yelping.

Fredricson (1950) found that confinement of an isolated puppy outside the home pen would produce high rates of vocalization in puppies 6 to 10 weeks of age. In a second paper (Fredricson, 1952) he showed that placing a second puppy in the box reduced the rate of yelping 50 per cent. or more, and that substituting a small box in place of the puppy had no appreciable effect. He also found a larger differential with puppies that were first placed in confinement with another puppy than with puppies first placed alone in the box. Causey (1956) obtained preliminary results with a small group of puppies which indicated that puppies isolated in a strange room showed more vocalization than those isolated in a familiar one.

From these studies a number of possible factors which affect distress vocalization might be considered, such as confinement, isolation, strangeness of the test environment, previous experience, and age. It also is clear that yelping may be a potential indicator of various sorts of emotional distress in the puppy. It was our purpose to explore the characteristics of yelping behaviour: (1) by determining if reliable differences in yelping could be secured which would reflect imposed variables of restraint and isola-

tion, and (2) by determining if longer and continued exposure to the distress producing situation resulted in a decline in yelping. Two experiments were made.

Subjects and Methods

Twenty-nine hybrid and 6 pure bred puppies from 3 to 6 weeks old were used as subjects. Details of breeding, sex, and age are shown in Table I.

The first experiment dealt with the effects of restraint, using yelping as the major measure of distress. Twenty-three puppies from 3 hybrid litters 4 weeks of age were randomly assigned into two basic groups: Restraint (R) or Non-Restraint (NR). We added the variables of being alone (A), or together with a litter-mate (T), giving us four conditions under which to measure yelping: (1) alone in pen (NR-A); (2) alone in restraint box (R-A); (3) together with a litter-mate in pen (NR-T); and (4) together with a litter-mate in restraint box (R-T). The puppies in each group were tested each day for 5 minutes in one of the following orders: ATTA and TAAT. The series was repeated once.

Prior to testing, the mother and all puppies were removed from the home cage and placed in a large cart which was moved out of sight of the cage. The puppies to be tested were then replaced singly or in pairs in the home cage and allowed to run around freely, or were placed inside the restraint box. The number of yelps was recorded by the observer, who stood quietly nearby and counted them with a hand counter. When puppies were tested in pairs it was easy to determine which puppy was making the noise by observing characteristic mouth and head movements.

For the second experiment, 12 puppies, aged 3 to 6 weeks, were randomly assigned to the extreme groups of the first set of experimental conditions: Restraint-Alone (R-A) and Non-Restraint-Together (NR-T). Testing duration was 10 minutes with yelping scores tallied at the end of each 5-minute period. One trial a day was made for 10 consecutive days.

The restraint box was made of wood in a triangular shape: $17\frac{1}{2}$ in. long, $9\frac{3}{4}$ in. across the front, and $6\frac{1}{2}$ in. deep. The lid was made of wire

Table I. Characteristics of Subjects

Experiment	Number in litter	Sex, M/F	Parentage	Weight range Kg.	Age in days at first test
1	7	4/3	Scottie—Cocker—Beagle x Elkhound—Basenji	3·76—4·50	28
	8	2/6	Scottie—Beagle x Schipperke—Cocker	2·00—4·38	27
	8	4/4	Scottie—Beagle—Schipperke x Scottie—Dachshund—Beagle	2·00—4·08	28
2	3	3/0	Pug—Dachshund x Pug—Dachshund	1·00—1·16	21
	1	0/1	Cocker Spaniel	1·46	38
	2	0/2	Cocker Spaniel	2·01—3·20	44
	3	3/0	Cocker Spaniel	1·54—1·76	25
	3	1/2	Cocker x Basenji	1·44—1·78	38

mesh. This material also covered the front, so that the restrained puppy was always able to "see" many familiar home objects. The majority of the home pens were the standard weaning pens, approximately 6 feet square with open wire fronts. The cocker spaniel puppies were reared in large nursery rooms, 10 ft. × 17 ft. in size, and enclosed on all sides.

Other indications of distress recorded during the experimental trials included degree of activity, defaecation, and urination.

Results

The results of the first experiment, in which yelping was measured under each of four experimental conditions, are shown in Table II and Fig. 1. The major variables were the effects of restraint against non-restraint and being confined alone against together with a litter mate. In addition, the dogs were tested in one of two orders, starting either under the alone condition or under the together condition of confinement.

Because of the unequal numbers of subjects (6 puppies in each cell except the NR-T first, where there were 5) an analysis of unweighted means was used. In this procedure the mean for each of the 8 cells was computed and an analysis of variance was performed on these means. The two-error term sums of squares (subjects within cells and A × S) were computed directly from the raw scores and were then reduced to a mean value by dividing by the harmonic mean. The method

is essentially the same as that described by Walker & Lev (1953, pp. 381-382).

The analysis shows that yelping was significantly more frequent when the subjects were restricted than when non-restricted (p<0·01),
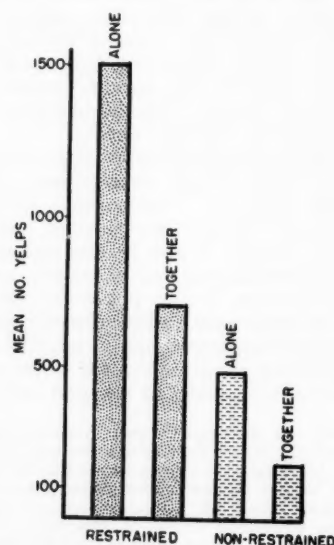
Fig. 1. The mean number of yelps for each of the groups: restraint and non-restraint under the alone and together conditions.

Table II. Number of Vocalizations Under Conditions of Isolation and Restraint

Condition			ATTA		TAAT	
			Alone	Together	Alone	Together
Restraint	Mean		1506·5	785·0	1480·3	636·6
	SD		247·8	290·7	326·5	454·5
	N		6		6	
No-Restraint	Mean		460·1	83·5	470·6	294·0
	SD		292·0	287·0	89·8	436·4
	N		6		5	

and that the alone condition brought about a significantly greater number of yelps than the together condition (p. <0.01). Further, these two variables interacted significantly at the 0·05 level. Fig. 1 shows that the combined effects of being alone and in a restricted environment apparently acted to increase differentially the number of yelps. The order of testing was not significant, nor did this variable interact with either of the other two main effects either singly or in combination.

The nature of the observed effects is clear. Undisturbed puppies in the home cage with their mother and litter-mates ordinarily show little or no distress vocalization. The puppies which were placed alone in the pens gave some yelps in every case. In the non-restrained puppies the range was from 7 to 687 in a 5-minute period. In the restrained puppies the range was from 485 to 969. Being alone in the home pen produces a definite reaction, though not as great as being alone under other conditions.

Placing a litter-mate with the puppy reduces the amount of emotional distress as indicated by yelping, but does not bring it back down to zero in all cases, as does restoring the mother and all litter-mates. The effect of adding a companion is highly significant statistically. In the 12 re-strained puppies every one showed more yelps alone than when together with a littermate.

Restraint produces similar highly significant effects. Each animal in the restrained group can be compared with a non-restrained litter-mate tested in the same way. In every one of the 11 pairs the restrained puppy showed more total yelps than the non-restrained litter-mate, whether the testing was done alone or together. There is little doubt that restraint produced large and significant increases of yelping.

The relationship between the experimental factors is obviously not a simple additive one, as can be seen in Fig. 1. In non-restrained puppies, adding the second puppy reduces the average number of yelps by 286. The effect of the second puppy upon a restrained littermate is to reduce the average number of yelps by 783. The second puppy has more than twice the effect that it had under the first condition. However, the per-centage of reduction compared to the total is approximately the same, being 60 per cent. for the non-restrained puppies and 52 per cent. for the restrained ones. In other words, the presence of a second animal appears to reduce the over-all excitement by a proportional amount rather than by an absolute amount, and this agrees with Fredericson's (1950; 1952) results. This relation-ship probably explains the interaction effect brought out by analysis of variance.

The second experiment was designed to study the effect of adaptation to the two extreme con-ditions of Experiment 1, viz. restricted-alone against non-restricted-together. Four subjects under the first condition and eight under the latter were given one 10-minute test per day for 10 consecutive days. The means and standard deviations are presented in Table III, and Fig. 2 is a graphic presentation of the findings. The highly significant difference between the two groups confirms the findings of the first experi-ment that the conditions of restricted-alone and non-restricted-together bring about major differ-ences in yelping behaviour. The significant trials effect indicates that yelping decreased over the 10-day interval, presumably due to adaptation to the experimental conditions. The lack of a significant interaction between groups and trials shows that this adaptation was the same for both groups,

Table III. Results for Experiment 2

Condition		Trials									
		1	2	3	4	5	6	7	8	9	10
R—A (N=4)	Mean	1128·75	1348·75	1182·00	1167·25	1154·50	1090·75	1041·75	1017·75	884·00	920·25
	S.D.	341·22	399·45	390·81	601·34	567·18	725·12	297·33	392·83	572·99	512·93
NR—T (N=8)	Mean	348·25	281·87	397·50	439·00	214·87	234·12	290·12	50·00	67·00	128·00
	S. D.	478·37	296·86	450·73	391·04	254·76	294·56	308·24	43·80	110·40	147·54
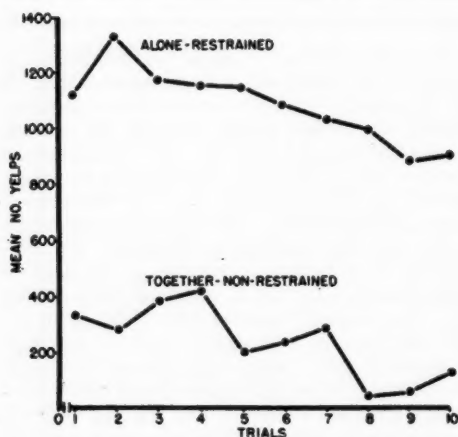
Fig. 2. The mean number of yelps for 10 trials for the Alone-Restraint and the Together-Non-restraint groups.

As a check upon the adaptation hypothesis, the number of yelps emitted during the first 5 minutes of testing was compared to the number emitted during the last 5 minutes for trials 1 and 10. Significant differences at the 0.05 level (one-tailed tests) were found for each of the trials.

Discussion

The results of the first experiment demonstrate that the yelping behaviour of puppies can be produced by placing them alone in the home pen. Restraint added to isolation markedly increases the amount of yelping. Adding a littermate has the effect of reducing the total amount of yelping by approximately 50-60 per cent. These findings confirm those of Fredericson (1950; 1952) and, in addition, indicate that physical restraint is one of the factors that contributes to emotional distress. Support is also given to the preliminary finding of Causey (1956), that isolation produces less distress in the home pen than in unfamiliar surroundings.

As can be seen from Table II, yelping behaviour is highly variable between individuals. The behaviour of individual puppies is much more consistent, there being "high" yelpers and "low" yelpers. Individual reaction to the experimental factors is consistent. The possibility that the results could be obtained by accident of sampling of genetically different individuals is extremely small. That such clear-cut results can be obtained with a group of hybrids may indicate that the phenomena are characteristic of all dogs rather than one particular breed.

The finding that the order of testing produces no significant effects is contrary to Fredericson's results that a puppy first confined with a littermate away from the home pen is less upset by subsequent isolation in the same situation. This difference is probably explained by the short period of testing used in the present experiment (5 minutes). The amount of yelping dies down chiefly in the second 5 minutes (Experiment 2, Fig. 2), and Fredericson's puppies were tested for 10 minutes.

The second experiment chiefly dealt with the effects of the duration factor on the yelping behaviour shown under the two extreme experimental conditions. Over the period of 10 trials, the mean number of yelps for both the restrained alone and non-restrained-together groups showed a significant though gradual decrease. It is more likely that this decrease is due to adaptation to the situaton and learning rather than to maturation, since the decrease was obtained within trials as well as between trials. The most likely explanation is that the puppy learns that it will be released after a short time, and hence becomes less disturbed emotionally.

Defaecation and urination were not important

dependent variables in either experiment. Yelping was greatest in the stress condition of R-A, but of 40 tests made with the restraint box, in only two were there signs of eliminative behaviour. This behaviour was exhibited more frequently by puppies in the least stress producing situation, that of NR-T. Among restrained puppies there was an observable amount of increased activity, which usually included biting, licking and clawing the front mesh of the restraint box. Play behaviour was exhibited often (and only) by puppies in the NR-T condition.

The question might be raised as to whether or not the conditions of restraint and isolation are innate releasing mechanisms. The reaction of yelping or whining is a primary one, appearing at birth, and some of the adequate stimuli or releasers are pain, cold and hunger. However, the reaction of excessive vocalization does not appear in older puppies which have been reared alone in small boxes from before the time of the critical period of socialization (Fisher, 1955). Such animals are both restrained and isolated. We are dealing here with something more complex than a simple IRM: an interaction of environmental situations, organization of behaviour through learning, and innate organisation of behaviour. The yelping response is modifiable by hereditary factors (Scott, 1957), and it is likely that there is a tendency to develop this response to isolation and restraint under normal conditions of rearing.

As et-epimeletic or care-soliciting behaviour, the yelping of puppies is equivalent to the distress vocalization of other animals. Such behaviour offers many experimental possibilities, both as a measure of the strength of social relationships involved in imprinting and primary socialization, and as a measure of emotional distress in young animals, including human babies, where separation from the mother has been found to have important psychological effects (Bowlby, 1951). Further, yelping behaviour may prove to be a reliable and sensitive measure of a variety of imposed experimental variables.

Summary

This study dealt with the effects of restraint, isolation and companionship on the yelping behaviour of puppies. Thirty-five puppies of various complex hybrid and pure breeds, aged 3 to 6 weeks, were used. The condition of restraint was produced with a small triangular wooden box, open at the top and front, which was covered with wire mesh. All tests were in the home pen, and yelps were recorded with a hand tally counter.

In the first experiment the effects of restraint (R) against non-restraint (NR) were tested as modified by the condition of alone (A) and together (T). Twenty-three puppies, aged 4 weeks, were randomly assigned to one of the basic groups of R or NR. They were then given a series of 5 minute tests, ATTA or TAAT, under the basic conditions of R or NR. The yelping behaviour of the restrained (R) group was found to be significantly higher than the non-restrained (NR) group, and the alone (A) condition produced more yelping than the together (T) condition. Both of these differences were significant beyond the 0·01 level. Both isolation and restraint increase yelping. Adding a companion reduces yelping by 50-60 per cent.

The second experiment was performed with twelve puppies, aged 3 to 6 weeks, randomly assigned to the extreme conditions of the first experiment, those of R-A and NR-T. The effects of test duration upon yelping behaviour was studied with 10 trials, one per day, of 10 minutes each. Statistical analysis of these results demonstrated that there was a similar and significant decrease (0·01 level) in the mean number of yelps in both the R-A and the NR-T groups. Comparison of means for the first 5 minutes against the second 5 minutes made on trials 1 and 10, showed a decrease in yelping behaviour significant at the 0·05 level. It was concluded that repetition of the experience reduces yelping, probably because of learning and adjustment to the situation.

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THE RELATION OF CASTRATION, ANDROGEN THERAPY AND PRE-TEST FIGHTING EXPERIENCE TO COMPETITIVE AGGRESSION IN MALE C57 BL/10 MICE*

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Introduction

Aggressiveness is a characteristic of behaviour that is readily identified in the single instance of observation but hard to define in the general case. A wide variety of responses have been called aggressive, but the compilation of a definitive set of differentiating symptoms is yet to be achieved. Laboratory studies of animal behaviour have yielded a dichotomous classification: spontaneous aggression and competitive aggression. Spontaneous aggression applies to behaviour for which the only identifiable stimulus appears to be the physical proximity of another animal; competitive aggression refers to behaviour associated with the presence of a goal presumed to be attractive to several subjects at the same time. Behind this dichotomy are two opposing orientations: the constitutional and the situational. The former treats aggressiveness as a general biologically-based trait and is founded on reports of species and sex differences in behaviour, and of the effects of castration and replacement therapy. The latter regards aggressiveness to be an acquired mode of reacting to frustrating circumstances and is derived from observations of competitive fighting in prepubertal animals, the development of social orders, situation-specific behaviour, and displaced aggression.

But to account for aggressive behaviour in terms of one bias to the exclusion of the other is disarming simplification. On the one hand, not all members of an aggressive species are aggressive, nor are aggressive members aggressive on all occasions. Though it is commonly held that the male is the dominant sex, there are species in which the female displays this role. While androgen therapy has been reported to restore pre-castration aggression level in mice (Beeman, 1947a; Bevan, Levy, Whitehouse & Bevan, 1958) massive doses appear to have a suppressive effect (Bevan, Levy, Whitehouse & Bevan, 1958;

Bevan, Bevan & Williams, 1958). While androgen injections will induce fighting in prepubertal males (Levy & King, 1953), it has no effect on castrate females (Tollman & King, 1956), and castrate male starlings appear to maintain their pre-castration social order, though given individually differing doses of testosterone (Davis, 1957). Meanwhile, though an animal's history of wins and losses relates to the level of aggressiveness it displays (Ginsburg & Allee, 1942), the age at which fighting experience first occurs (Fredericson, 1957) and the strain to which it belongs—even for competitive behaviour (Doner, Inman & Davis, 1952)—are important.

The aim of the present experiment was to select one type of aggressive behaviour—competitive fighting—and to explore the relative importance of representative variables of the two broad classes associated with aggressiveness by varying both within a single design. This allows not only a direct comparison of the two classes, but also an assessment of their possible interactions. Thus one may ask not only if differences in endocrine status and differences in pre-test experience both influence competitive fighting, but also if difference in endocrine status prior to specific pre-test experience influences the effect of the latter upon test performance, or, conversely, if a change in endocrine status subsequent to training influences the role of the experiential variable in fighting.

Method

Subjects

Seventy-two male C57 B1/10 mice, supplied by the Roscoe B. Jackson Memorial Laboratory, were used as subjects. These animals arrived at our laboratory at 35-40 days of age, and were immediately isolated in individual cages. None were handled except for surgery, replacement therapy, and to be placed in the test apparatus.

Apparatus

The test compartment consisted of an opaque sided box, 9 in. × 10 in. × 5½ in., with plexiglass

top and grid floor. The inside walls were lined with clear plastic to prevent climbing. A small platform $\frac{3}{4}$ in. × $\frac{1}{4}$ in. was mounted at the centre and one inch above the grid. The grid was charged by a 100 μa direct current, 240 pulses per second, from a Thomas Electronic Stimulator.

Design

The 72 animals were first randomly divided into four replication groups of 18 animals each. Within each group, the aggressiveness scores of each animal were based on observations of its behaviour in the presence of every other animal once. Each of the major variables, androgen status and pre-test training, was presented in three ways. One-third of the animals were maintained as normals, one-third were castrates, and one third were castrated and then placed on replacement therapy. In order to examine the relationship of the time of castration relative to training upon test behaviour, one half of both the castrate and the castrate-with-therapy groups were operated prior to aggressiveness training and one half after training. The three conditions of pre-test experience used were training in winning, no training, and training in losing. One-third of each replication was subjected to each condition. The design for each replication group is schematized in Table I.

Operative Procedure

Within one week of arrival all animals designated as pre-training castrates were subjected to one-stage bilateral castration. All post-training castrates received similar treatment immediately upon completion of training. One week after operation, all animals selected for replacement therapy were placed on a programme of weekly intramuscular injections of 1 mg. of depotestosterone cyclopentylpropionate in oil.

Training Procedure

All animals were first trained individually to mount the small platform at the centre of the test compartment to escape the electrified grid. The criterion for learning was three successive trials with latencies of five seconds or less. After criterion was reached each received three additional trials per day for three days. Meanwhile, 12 additional animals were trained in the platform response and then subjected to a round-robin series of competitions for access to the platform. From among these the most aggressive and least aggressive were selected to be used as "trainers" with the animals of the replication groups. The animals designated for experience at winning were then each placed on the electrified grid for five minutes each day on seven consecutive days and allowed to command the platform in the presence of the submissive trainer. Conversely, the animals designated for experience at losing were placed on the grid and required to accede the platform in the presence of the aggressive trainer. The control (no training) animals were kept in isolation during this period.

Test Procedure

Tests were begun two weeks after castration of the post-training animals. Each animal was paired for five minutes daily with another member of the replication group to which he had been assigned and allowed to compete for access to the escape platform. These pairings were continued until he had met every other animal in his replication once.

Test behaviour was evaluated by means of the following measures:

1. *Latency*. This is the time interval, in seconds, between onset of shock and the first successful attempt to mount the platform. If an

Table I. Experimental Design for Each Replication Group. Numbers in cells represent animals.

Androgen status		Type of pre-test experience		
		Control (no training)	Winning	Losing
Normals		2	2	2
Castrates	Pre-training	1	1	1
	post-training	1	1	1
Castrates with therapy	Pre-training	1	1	1
	post-training	1	1	1

animal failed to mount during the test period it was assigned a latency of 300 seconds.

2. *Duration*. This is the total time in seconds during which the animal maintained itself on the platform.

3. *Intensity*. First, each animal was rated for intensity on two scales: The on-platform intensity scale and the off-platform intensity scale. The on-platform scale consisted of five categories varying from (1) *mouse yields platform immediately when pushed by opponent*, through (5) *mouse vigorously resists opponent by pushing, crowding, or butting; it may bite opponent*. If the mouse never mounted the platform during a test, it was scored "0"; if it remained on the platform, but was never challenged, it was scored "x". The x values were not included in the computation of the intensity scores. The off-platform scale also consisted for five categories. These varied between (1) *mouse remains off grid, only occasionally (less than seven times) approaching platform; it does not attempt to mount platform, but may try other means of avoiding shock*, and (5) *mouse vigorously tries to unseat opponent with frequent butts and pushes; it may unseat opponent; it may bite opponent*. If the animal remained on the platform, unchallenged, during an entire test, it was scored "0", and this score was not included in the computation of the intensity score.

The intensity score was next derived by totalling all of the on-and-off platform ratings of four and five, and dividing by the total number of possible occasions on which the score could have been received. For the 17-trial round-robin the divisor was 34 (17 times 2 ratings for each trial) minus the number of on-platform x-ratings and off-platform zero ratings: Thus

$$I = \frac{\Sigma\, 4\text{'s} + \Sigma\, 5\text{'s}}{34 - (N_x + N_o)}$$

4. *Relative seminal vesicle weight*. All animals were sacrificed within a day or two of the last test. The present measure was obtained by determining the ratio between organ weight in millgrams and terminal body weight in grammes. Seminal vesicle weight is probably the most widely used indicator of androgen level in infrahuman mammals. (cf. e.g. Chai, 1956). Findings such as Beeman's (1947a) of correlative changes in organ size and level of aggressiveness following castration and subsequent replacement therapy have led to the assumption that organ size is a valid index of level of androgen influence upon the nervous system. At the same time, the small Spearman r's between this measure and the several measures of behaviour obtained in the present experiment (Table III) suggest the fact that the sensitivities of the ductus deferens and of the nervous system are not the same. Unfortunately, available facilities precluded securing other physiological indices such as measurement of the thickness of the epithelium of the vesicles.

Results

Relations Among Measures

The data for the fifteen groups representing the several conditions of androgen status and pre-test experience are summarised in Table II. Intercorrelations between measures are presented in Table III.

Inspection of Table III indicates that all of the behavioural measures are significantly interrelated and that the degree of relationship varies from marked to very high. Moreover, a relationship between physical status and behaviour is reflected by the coefficients involving these two classes of variables. While none indicate more than a moderate degree of correlation, all but one are significant.

Physical Status

Two analyses of variance were performed on the body weight data. The first, omitting the normals, was performed to see whether time of castration and its interaction with therapeutic regimen had any effect upon this factor, since body weight relates in a significant degree to aggressiveness, probably by virtue of its relation to an animal's history of wins and losses. The interaction was not significant (F<1·00), nor was the castration-time source of variance (F=3·95, df=1/44, P>·05). Inspection of Table II indicates the castrates with therapy to be the heaviest groups, the normals next heaviest and the castrates without therapy the smallest animals. The second analysis, a simple F test, bore this out (F=16·96; df=2/69; P<·001). The subsequent Tukey gap test (1949) revealed a reliable gap between each pair of means in the order (significant gap=1·00, P=·05).

The androgen status of the several groups was assessed by comparing relative seminal vesicle weights. Because the distribution of these ratios was skewed, the square-root transformation was applied before statistical analysis. A two-way classification analysis of variance involving the castrate groups revealed a significant castration-time by therapy status inter-action

Table II. Mean Scores for Fifteen Experimental Groups on the Several Measures of the Present Experiment*

Androgen status		Type of pre-test experience					
		Controls		Winning		Losing	
Normals		L	92·18	L	50·75	L	113·79
		D	116·02	D	180·82	D	131·36
		I	1·11	I	1·76	I	1·30
		W	27·40	W	27·50	W	27·30
		S	2·10	S	2·22	S	2·18
Castrates	Pre-training	L	164·50	L	93·18	L	84·63
		D	60·25	D	143·62	D	136·26
		I	·84	I	1·08	I	1·36
		W	26·00	W	25·00	W	27·00
		S	·16	S	·53	S	·15
	Post-training	L	188·75	L	50·38	L	175·09
		D	55·26	D	193·72	D	76·00
		I	·49	I	1·62	I	·71
		W	25·30	W	26·40	W	24·30
		S	·51	S	1·06	S	·82
Castrates with therapy	Pre-training	L	67·12	L	55·88	L	105·66
		D	136·65	D	188·35	D	129·46
		I	1·39	I	1·78	I	1·48
		W	28·80	W	29·20	W	29·00
		S	2·29	S	3·30	S	2·83
	Post-training	L	144·08	L	35·51	L	130·04
		D	108·94	D	215·93	D	128·70
		I	1·40	I	2·11	I	1·26
		W	27·60	W	28·80	W	27·70
		S	2·56	S	2·40	S	1·70

* L=latency in seconds.   D=duration in seconds.
I=intensity.   W=terminal body weight in grammes.
S=relative seminal vesicle weight $\times$ 10^3.

(F=8·37, df=1/44, P<·01). The Tukey gap test applied to the cell means (significant gap =2·12, P=·05) further suggested no difference between the pre-training and post-training groups receiving therapy. These, however, tended to have relatively heavier seminal vesicles than the post-training no-therapy groups. In turn, the latter differed significantly from the pre-training, no therapy animals. Comparison of the normals and the therapy groups combined yielded no evidence of a significant difference (t = ·76, df = 46, P>·05). Meanwhile, a comparable comparison

Table III. Product-moment Intercorrelations Between the Several Measures Used in the Present Experiment.

	L.	D.	I.
D.	—·903**		
I.	—·754**	·799**	
W.	—·260*	·335**	·449**
S.	—·199	·265*	·239*

*Significant at ·05 level.
**Significant at ·01 level.

with the no-therapy group indicated that the castrates had relatively lighter seminal vesicles (Since the variances of the normal and two sets of no-therapy animals were heterogeneous, both t-prime and t were computed (Cochran & Cox, 1950, 92). Normals vs post-training no-therapy: t-prime=2·03, t=9·45, df=34, P<·05; normals vs pre-training no-therapy: t-prime=2·04, t=6·17, df=34, P<·01).

Readiness to Engage in Aggressive Behaviour: the Latency Score

Because of positive skewness, the latency scores were subjected to the square-root transformation before further evaluation. Of first concern was whether or not time of castration relative to training was significantly related to readiness to respond. This question was resolved by comparing the pre-and post-training castration groups not receiving therapy. Comparison yielded no significant difference (F=2·00, df=1/12, P>·05). Thus it would appear that training did not differentially affect animals of different endocrine status, nor once it had been accomplished, was it subject to influence by changes in endocrine status. The possible influence of instituting the therapeutic regimen before as contrasted to after training was next examined. Here again there was no evidence that time made a difference (F<1·00).

Meanwhile, an overall two-way analysis of the latency data indicated several things. Type of pretest training experience reliably influenced readiness to compete for access to the platform (F=9·92, df=2/63, P<·001). The Tukey gap test (gap=1·95, P=·05), further suggested the losers and the animals which had received no pre-test competitive experience were slower initiating fighting than those trained in winning. In contrast, neither androgen status (F=2·16, df=2/65, P>·05) nors its interaction with pre-

test experience (F<1·00) contributed significantly to the latency score.

Persistence in Aggressive Behaviour: The Duration Measure

The same sequence of analyses performed on the latency scores was also performed on the duration measures. Analysis of the scores of the castrate groups revealed neither the castration-time by type-of-training interaction (F=2·94, df=1/12, P<·05) nor that of castration-time by therapeutic regimen (F<1·00) to be significant. Similarly, the castration-time main effects F's did not exceed 1·00.

Meanwhile, the double classification analysis applied to all groups indicated pre-test training experience, and, in the case of the latency scores, androgen status both to be reliable sources of variance (F$_{experience}$=13·75, df=2/63, P<·001; F$_{androgen}$=3·24, df=2/63, P<·05). Meanwhile, the Tukey gap test applied to the ordered means for androgen status revealed neither a significant gap nor the presence of a straggler. A significant gap did appear between the average of the winning groups and the two remaining training conditions (Gap=33·50, P=·05).

The significant F for androgen status, even though left unclarified by the absence of significant gaps, would appear to be a novel finding for competitive aggression, since the previous literature has given little indication of its operation. It further raises the interesting question of how androgen status exerts its influence on competitive fighting. Several possibilities suggest themselves: Through its effect on the animal's vigour, strength, and body size. Table III has indicated a reliable positive correlation between duration and body weight and it is commonly accepted that difference in body size may be related to androgen status. Accordingly, an analysis of covariance was performed on the duration data to correct for differences in body weight. When this was done the signicant F of 3·24 between androgen treatment groups reduced to less than 1·00. Thus it would appear that any effect that androgen status has upon competitive aggressiveness is exercised through its influence on body weight.

Vigour of Aggressiveness: The Intensity Score

Because of positive skewness, and in order to eliminate zeros from the analysis, the data was increased by a constant and transformed to logarithms before analysis. Again, as in the case of the two previously described behavionral

indices, when the scores of the castrate groups were analysed, no significant castration-time by type-of-training or castration-time, by therapy-status interactions ($F=3\cdot84$, $df=1/13$, $P>\cdot05$, and $F>1\cdot00$) were found. Similarly, time of castration relative to time of training appeared to have no effect on test performance.

When the data of the normals were analysed along with those of the operated-treated animals both androgen status and type of pretest experience proved to be significant sources of variance ($F_{androgen}=4\cdot39$, $df=2/63$, $P<\cdot05$; $F_{experience}=5\cdot01$, $df=2/63$, $P<\cdot01$). In the case of androgen status, the Tukey gap tests did not indicate a reliable gap between ordered means although the computed z suggested the castrate-no-therapy group to be a straggler. In contrast, for the experiential variable, the Gap test gave the same ordering as for latency and duration—winners significantly more intense than losers or controls (Gap = $\cdot0530$, P = $\cdot05$).

The significance of the reliable androgen status source of variance was further examined by analysis of covariance correcting intensity for body weight. Here, as in the case of duration, a significant F ratio of $4\cdot39$ was reduced to less than $1\cdot00$. Thus androgen status influenced aggressiveness through its relation to body-weight.

Discussion

The results of the present experiment may be briefly summarized as follows: Competitive aggression varied as a function of two classes of variables: type of pre-test fighting experience and androgen status at time of test. Inspection of mean squares and probability values for the associated F-ratios suggest the former to be the more influential variable. Animals that were allowed to dominate the escape platform prior to the round-robin test series claimed the platform more quickly, kept themselves on it longer, and fought more vigorously than animals trained as losers or allowed no pre-test experience in competition for the platform. Meanwhile, no differences in test behaviour were detected between the latter two groups. At the same time, normal animals and castrated animals receiving replacement therapy, irrespective of pre-test experience, appeared to fight more vigorously and maintain themselves on the platform longer than castrates without further treatment. These last differences in performance, in turn, appear to be attributable to differences in body weight. Finally, whether castration is

performed before or after pre-test training is irrelevant.

The failure for variation in the temporal order of castration and pre-test training to influence test scores requires some comment. Averaging across type training of experience yields values that differ only slightly for pre-and post-training castration. In addition, since each cell in this portion of the design contains only four scores, the related variances are relatively large. The data thus are not in line with Beeman's report (1947 ab) that animals of the present strain castrated prior to initial test fail to fight while those castrated after some fighting experience persist in fighting after operation. Meanwhile, they do appear to be in line with her observation that age of castration is irrelevant if a period of sufficient length intervenes between orchidectomy and test. Unfortunately, in the present design, age of castration and castration-training order are confounded variables.

The superiority of the pre-test winners is consistent with the reports of Ginsburg & Allee, (1942) and of Kahn (1951). However, the lack of difference between the controls and the losers needs clarification. One possible explanation is that acquired dominance behaviours generalize more readily than responses associated with submission. This possibility is supported by Seward's finding (1946) that white rats, when exposed to a non-aggressive opponent after a severe defeat, exhibited extinction of the fear responses made to the earlier aggressive opponent. Another reasonable speculation lies in the possibility that familiarity with the presence of another animal in the test situation may serve to counteract the inhibitive effects of prior losses to place the losers at least on an equal footing with the controls not allowed this familiarization.

The existence of differences between the performance of the castrates and the normal and treated animals would seem to be in line with Beeman's (1947a) and Bevan, Levy, Whitehouse & Bevan's (1958) findings for testosterone therapy and spontaneous aggressiveness. It would also appear to provide a qualification of Scott & Fredericson's conclusion (1951) that hormone effects are confined to spontaneous aggression. At the same time, it must be emphasized that the androgen effect is an indirect one, exerting its influence through its relation to the factor of body weight. The finding that time of castration is irrelevant for test performance supports Beeman's observations (1947b) and

precludes an extension of Uhrich's conclusion about this variable (1940) to the present situation.

Summary

The relative influence of both androgen status and pre-test fighting experience upon competitive aggression was studied with 72 C57 BL/10 male mice kept isolated in individual living cages except for testing. Twenty-four animals were castrated at 35-40 days of age. Half of these were placed on replacement therapy of testosterone propionate for the remainder of the experiment. After several weeks of isolation all animals were trained to escape shock by jumping to a small platform at the centre of the floor. One third were then forced to compete for the platform with an aggressive trainer, one third with a submissive trainer, and the final third received no competitive experience. After training, an additional 24 animals were castrated. Twelve of them received replacement therapy. Two weeks after training each mouse was placed in a round-robin test sequence of 17 pairings representing each combination of pre-test experience and androgen status.

Analysis of the data from 1224 bouts yielded the following results:

1. Time of castration relative to training had no effect on fighting in the competitive test situation.

2. Winning during the training series was associated with the most intense aggression in the test situation. Meanwhile, no reliable differences were demonstrated between losers and animals allowed no pre-test competitive experience.

3. Androgen status was shown to have some relationship to aggression, but this appeared to be evidenced through its effect upon body weight.

4. Of the two variables tested, androgen status and pre-test experience, pre-test experience appears to have been more influential.

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EOSINOPHIL RESPONSE TO AGGRESSIVE BEHAVIOUR IN CFW ALBINO MICE

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Introduction

This research investigates the fluctuations of eosinophil numbers in laboratory mice subjected to two aggressive behaviour situations. The relative reduction of eosinophil numbers from their normal levels and changes in adrenal weights are indicators of stress.

Many investigations have demonstrated pronounced reduction of eosinophil numbers in laboratory animals during and following various situations. Most previous work has utilized physical stressors such as venesection, hypoxia, cold temperature and injection of foreign substances (Halberg, 1953; Kostial *et al.*, 1958; Louch *et al.*, 1953; Speirs & Meyer, 1949). Recent work by Louch (1958) on natural populations of meadow voles has demonstrated eosinopenia and adrenal hypertrophy under conditions of high population density. Southwick (1959) using laboratory mice, found a reduction in eosinophil levels resulting from short-term behavioural disturbances. Eosinophil responses to different intensities of aggressive behaviour have, however, not been investigated.

In experimental animals, eosinopenia is known to be a response to increased adrenocortical activity (Forsham *et al.*, 1948; Speirs & Meyer, 1949). This, in turn, is a response to increased pituitary secretion of adrenocorticotrophic hormone (ACTH). The eosinophil response is sufficiently precise to serve as a bioassay method for cortisone and ACTH (Dumm & Ralli, 1954; Rosenberg, *et al.*, 1954; Speirs & Meyer, 1949), thus the relative degree of eosinopenia can be used as a quantitative measurement of the severity of the stressor.

Morphological changes of the adrenal gland have been studied more extensively than have eosinophil fluctuations. Numerous studies by Christian (1955a; 1955b; 1956), Christian & Davis (1956), Chitty *et al.* (1956), Clarke (1953), Louch (1958) and others have found that mice (*Mus musculus*), rats (*Rattus norvegicus*) and

voles (*Microtus pennsylvanicus*, *M. agrestis*, and *M. orcadensis*) respond to increased population density by adrenocortical hypertrophy. Southwick (1958) and Southwick & Bland (1959) however, did not find adrenocortical hypertrophy resulting from increased population density in house mice. Therefore it is felt that additional study of the effects of crowding is necessary using more sensitive indicators than adrenal weights.

It has been observed by Bland (1958) that four strange male CFW mice when brought into contact displayed vigorous fighting behaviour. This spontaneous fighting behaviour was reduced when two strange pairs of male and female mice were brought together (Bland, 1958). In the present study, population density was kept constant at four mice per cage but the sex ratio of the group was varied in order to bring about two intensities of aggressive behaviour.

Methods

In Experiment A 60 male CFW albino mice (*Mus musculus*) weighing 8 to 12 grammes (21 to 25 days of age) were individually isolated for three weeks in wire cages (7 in. × 9½ in. × 7 in.). At 9.00 a.m. on the morning of the 21st day after weaning the mice were weighed and placed into larger cages (16 in. × 9½ in. × 7 in.) in groups of four. Nine cages were so arranged. Controls remained individually isolated in their original cages. Purina Laboratory Chow and tap water were supplied *ad libitum*. Artificial light was supplied daily from 8.00 a.m. to 11.00 p.m., and the temperature was maintained between 70° and 80°F.

Eosinophil counts were taken 4 hours, 72 hours, 120 hours, 1 week, 2 weeks and 3 weeks after the mice were placed into groups of four. Control counts of mice remaining isolated were taken at the same times. The mice were weighed at each count. Since eosinophil levels in mice display a marked diurnal rhythm (Halberg & Visscher, 1950) all counts were taken between 1.00 p.m. and 4.00 p.m. The technique used to take the eosinophil counts was essentially that of

Speirs & Meyer (1949). The alteration consisted of changing the eosinophil diluent to:

> 5 ml. 0·1 per cent. Phloxine Solution.
> 0·5 ml. Diethylene Glycol.
> 2 drops of 0·5 per cent. Alconox (detergent)
> 15 ml. Distilled Water.
> 3·2 ml. Acetone.

Slightly reducing the amount of acetone ensured that no eosinophils were destroyed and reduction of all the reagents by one half of the quantities given by Speirs & Meyer was for the purpose of economy.

Levy counting chambers with Fuchs-Rosenthal ruling and 0·2 mm. depth were used. The mean number of eosinophils of the two chambers charged with each blood sample was more conservative for statistical purposes than the mean number of eosinophils per mm.³. Conversion to eosinophils per mm.³ is possible by multiplying by 6·25.

At the termination of the three week research period the animals were sacrificed and the adrenal glands of 24 mice in the groups of four and 16 of the isolated controls were immediately removed and weighed on an analytical balance. The testes were removed and placed in Bouin's fixative for subsequent weighing.

In Experiment B, 106 mice were isolated as pairs; one male and one female. After the three week isolation period 60 mice were placed in groups of four consisting of two pairs. The controls remained isolated as pairs. Eosinophil counts, adrenal weights and testes weights were determined for all males. Other than the introduction of females, the same general plan as Experiment A was followed.

Results

Experiment A

Four strange male mice when placed in a cage displayed intense fighting behaviour. Fighting began almost immediately upon contact and continued at a high level of intensity for about two hours. The fighting activity slowly diminished by 72 hours and sporadic fighting was observed to continue for about one week thereafter. By 72 hours social rank was readily discernable. The dominant individual characteristically isolated himself from the other three mice by retiring to one corner of the cage and usually concentrating the food pellets in his corner. The three subordinates seemed to be equally low in rank and huddled in a corner of the cage opposite the dominant. The subordinate mice were wounded to a varying degree about the tail and rump region. Four subordinate mice died (three in one group and one in another), presumably as a result of wounds. The dominant mice remained unwounded. This dominant-subordinate social rank behaviour persisted for the duration of the experimental period (3 weeks).

The grouped and isolated mice showed (Table I) approximately the same pattern of weight increase throughout the experimental period, with the weight gain of the grouped mice slightly exceeding that of the controls by the second and third week. The comparison of the mean body weights of the dominant individuals to the subordinate individuals (Table II) showed that the dominant was the heaviest mouse in its group in seven of the nine groups. In two groups the dominant mouse had the second highest weight.

Table I. Mean Body Weights (grammes) of Male Mice in Groups of Four and Individually Isolated Males.

	4 hr.	72 hr.	120 hr.	1 wk.	2 wk.	3 wk.
Grouped Males	20·2 (n=36)	20·9 (n=34)	21·6 (n=32)	22·1 (n=32)	23·1 (n=31)	25·6 (n=31)
Isolated Males (n=24)	20·3	21·4	21·5	21·9	22·4	24·5

Table II. Mean Body Weight (grammes) of Dominant Male Mice and Subordinate Male Mice.

	72 hr.	120 hr.	1 wk.	2 wk.	3 wk.
Dominant	21·6 (n=9)	22·0 (n=9)	22·2 (n=9)	23·3 (n=9)	26·1 (n=9)
Subordinate	20·2 (n=25)	21·5 (n=23)	22·0 (n=23)	23·0 (n=22)	25·5 (n=22)

The mean eosinophil count of the grouped mice was significantly lower than the mean eosinophil count of the control mice at 4 hours, 72 hours, 120 hours and at 1 week (Fig. 1). The most striking difference was exhibited at 4 hours after grouping where there was a 94·3 per cent. decline from the control mean. At 2 weeks there no longer existed a significant difference between the control and experimental means.
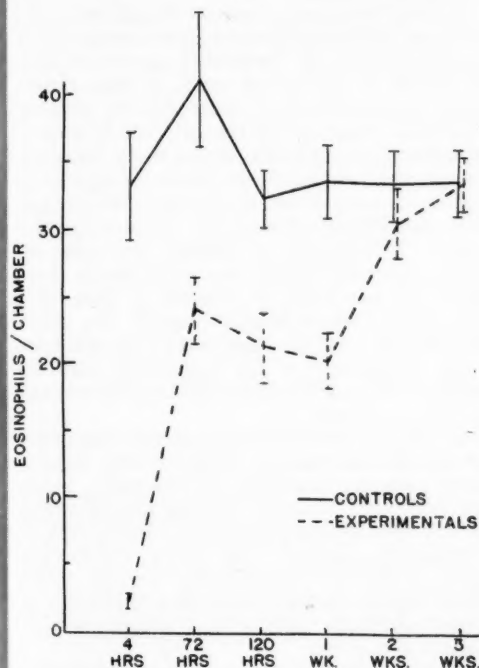
Fig. 1. Eosinophil levels of isolated males and groups of four strange males. Statistical significance occurred at 4 hours, 72 hours, 120 hours and at 1 week.

As is shown on Table III the adrenal glands of the grouped mice weighed significantly more than those of the isolated mice ($P = < ·001$). The relative adrenal weights for the grouped males was 0·170 mg/g. of body weight compared to 0·150 mg/g. for the isolated controls. The mean weight of the testes of the grouped mice was significantly lower than that of the isolated mice ($P = < ·05$).

A comparison of the eosinophil levels of the dominant to the subordinate mice in relation to the controls is shown in Fig. 2. Comparison of
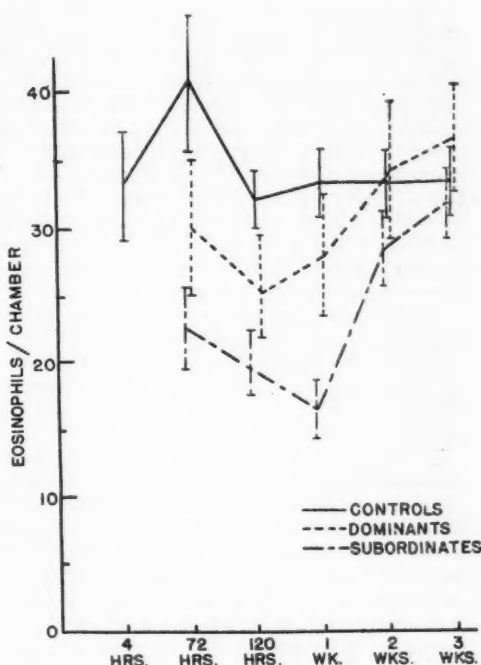
Fig. 2. Eosinophil levels of dominant and subordinate mice in groups of four with controls. Statistical significance occurred at 1 week.

the mean count at four hours was not possible because the social rank organization was not clearly indicated at this time. The mean counts of the dominant mice remained consistently higher than the mean counts of the subordinate mice. However, only at one week after grouping was there a statistically significant difference ($P = < 0.02$). The absolute adrenal gland weight of the subordinate mice was slightly heavier than that of the dominant mice but this difference was not statistically significant. When calculated on a relative basis the adrenal glands of the subordinate mice were also heavier (0·180 mg/g) than the dominant mice (0·165 mg/g).

Experiment B

Two strange pairs (one male and one female) when grouped displayed considerably less fighting than that displayed by the male populations in Experiment A. The mice spent more time investigating their surroundings and did not fight at initial contact. In two of the 15 groups of four

no fighting behaviour was observed within thirty minutes after grouping. In those groups where fighting occurred it was observed that only males initiated the encounter. Females were occasionally attacked but the encounter was of shorter duration than when the fighting occurred between two males. Fighting was sporadic and mild in all groups and was no longer observed after 72 hours. Slight wounds were observed on a few mice but no rump wounds were noted. Social rank behaviour was not observed. All four mice characteristically huddled in one corner when the sporadic fighting decreased.

Six females gave birth to litters: five in the isolated pairs and one in a group of four.

There were essentially no differences between the body weights of the males in the groups of four and the males of the paired isolates as shown in Table IV.

The mean eosinophil counts of the groups of four mice when compared to the mean eosinophil counts of the paired controls (Fig. 3) showed that only at 4 hours was there a significant difference between the means ($P = < 0.052$). Within 72 hours after grouping and for the rest of the experimental period there was no longer a significant difference between the means. There was no significant difference in either the adrenal weights or the testes weights (Table III).

Table III. Adrenal Gland and Testes Weight (mg.) of Mice at Third Week after Grouping.

	Adrenal wt.		Testes wt.	
	Mean	± S.E.	Mean	± S.E.
Isolates	3·8	0·049	166·6	5·226
Four males	4·5	0·048	150·8	4·554
One pair	4·0	0·055	156·3	3·405
Two pairs	3·9	0·084	155·7	2·153

Discussion

Groups of four strange male mice (Experiment A) displayed social rank behaviour similar to that described by Scott & Fredericson (1951)

in that one individual became dominant and the other three subordinate. The rapidity with which social rank behaviour became evident was correlated with the high intensity of aggressive activity shown by this group. The intense aggressive activity among the males closely resembled that described as non-competitive by Scott & Fredericson (1951). It is possible that the fighting was competitive in the sense of competing for rank.

Groups containing females (Experiment B), however, did not exhibit the characteristic social rank behaviour nor the intense aggressive activity found in groups of males. Collias (1944) states that among many vertebrates the presence of females increases the intensity and frequency of fighting among males. In this study, however, the presence of females decreased the aggressive activity among males. Carpenter (1934) found that male howler monkeys are not more aggressive in the presence of females. The same investigator (1942) found that male rhesus monkeys will not fight for females if there is a plentiful supply of females present. The above findings lead to the conclusion that there are large exceptions to the generality that the presence of females increases fighting among male vertebrate animals.

Southwick (1955) has shown that aggressive activity in semi-natural house mouse populations generally increases with increased population density; however, fighting activity varied greatly between some populations irrespective of population density. The latter observation agrees with the results of the present study. Southwick, in the same study, also found that intense aggressive activity was correlated with poor litter survival.

The most intense fighting in the present study was accompanied by the greatest eosinophil reduction which occurred four hours after grouping four strange males together (Fig. 1). Significant eosinopenia had disappeared by the second week indicating that a stressful situation was no longer present. In groups consisting of two pairs the eosinophil response was less pronounced and of shorter duration (Fig. 3) indicating less stress

Table IV. Mean Body Weights (grammes) of Male Mice in Groups of Two Pairs and Isolates of One Pair.

	4 hr.	72 hr.	120 hr.	1 wk.	2 wk.	3 wk.
Grouped (n=30)	20·3	20·8	20·9	21·2	23·3	23·1
Isolated (n=22)	20·8	21·3	21·5	21·7	22·7	22·7
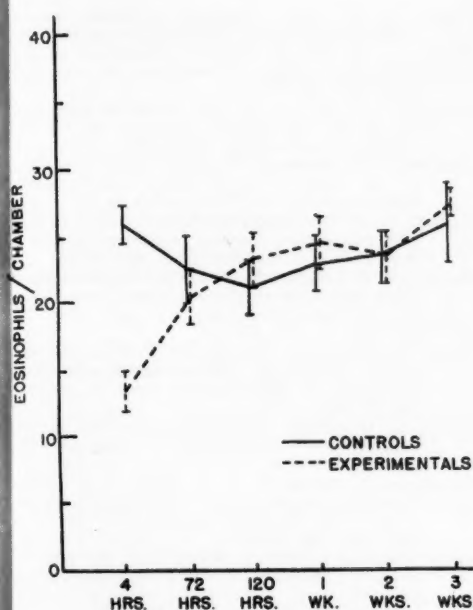
Fig. 3. Eosinphil levels of paired isolates and groups of two pairs. Statistical significance occurred at 4 hours.

to be present in these groups.

Southwick (1958), in a study of natural populations of house mice in English grain ricks found no correlation between population density and adrenal size. Southwick & Bland (1959) similarly found no correlation between population density and adrenal size in a laboratory study of albino house mice. They did, however, obtain adrenal hypertrophy in severely wounded individuals and in males grouped with females. The former agrees well with the data of this study, but the adrenal hypertrophy of the males grouped with females is in direct contradiction to the data of this study. The paired mice of Southwick & Bland's study were sacrificed one week after grouping. It is conceivable that an initial hypertrophy occurred during this week that would have been followed by a regression of adrenal size in the second and third weeks. This theory is supported in part by the eosinophil counts which indicate an initial eosinopenia followed by normal eosinophil counts for two weeks.

The significant increase in adrenal weights of the grouped mice in Experiment A (Table III)

agrees with the results obtained by numerous investigators (Christian, 1955b; Clarke, 1953; Louch, 1958). A significant decrease in testes weights accompanied the increase in adrenal weights as was also found by Christian (1955b). In Experiment B an increase in adrenal weights and a corresponding decrease in testes weights was not exhibited (Table III). This difference in results is thought to be due to the difference in aggressive activity shown by the two groups. Hence, adrenal hypertrophy was apparently a response to stress caused by severe fighting activity and not to population density per se.

Both Davis & Christian (1957) and Southwick & Bland (1959) found subordinate mice to have larger adrenals than dominant mice. A similar but non-significant difference occurred in the present study. The consistently higher eosinophil level displayed by the dominant mice supports the above findings and leads to the conclusion that subordinate mice are under relatively greater behavioural disturbance than dominant mice. A complicating factor is that the subordinate mice were severely wounded whereas the dominants remained unwounded.

Summary

Two populations of CFW albino mice of the same density (four per cage) but of different composition were set up in order to create two intensities of aggressive behaviour while maintaining a constant population density. One group of male mice was weaned at 21 days, individually isolated for 21 days, and then placed into groups of four. Isolated individuals remained as controls. The second group of mice was weaned at 21 days, isolated as pairs (one female and one male) and then placed into groups of four consisting of two pairs. Isolated pairs remained as controls.

Isolated male mice when placed in groups of four displayed intense aggressive activity which resulted in significantly depressed eosinophil levels for one week after grouping. There was a 94·3 per cent. decline in eosinophil levels 4 hours after grouping. Adrenal hypertrophy and decreased testes weights also occurred. Social rank behaviour was rapidly organized in these groups. The dominant individual consistently maintained a higher eosinophil level than the subordinates; this difference, however, was significant only at one week after grouping.

Groups containing females displayed mild aggressive activity which resulted in a significantly depressed eosinophil level (53 per cent. de-

cline) only at four hours after grouping. No significant adrenal or testes weight differences occurred. These results indicate that decreased aggressive activity results in decreased stress. Social rank behaviour was not shown in these groups.

The intensity of aggressive behaviour displayed by a population may be of greater significance than population density alone in influencing population dynamics in house mice.

Acknowledgments

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A QUANTITATIVE METHOD FOR THE ANALYSIS OF EXPLORATORY BEHAVIOUR IN THE RAT

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Introduction

The nature of variable behaviour has been the subject of much recent research and conjecture. The recent review of Barnett (1958b) has summarized and correlated the work of Montgomery (1951; 1952; 1953; 1954; 1955; and with Monkman, 1955), Berlynne (1950; 1951; 1955; and with Slater, 1957), Thorpe (1956) and Barnett (1956; 1958a). Many other workers have made valuable, but indirect, contributions to the understanding of behaviour which has variously been interpreted as appetitive, investigatory, spontaneously alternating, attention to change and exploratory.

Barnett (1958b) has pointed out that exploratory behaviour is "a behaviour pattern in its own right" (page 296). It appears to have the following characteristics which distinguish it from other forms of variable behaviour.

1. It is a form of variable behaviour in which there is no identifiable ultimate consummatory phase which is goal directed. Nor is there any special engendering internal state of the type which underlies other activities in the absence of external stimulation.
2. It takes the form of a series of qualitatively similar, alternating or variable investigations of the environment which is evoked by variety or change in it.
3. The external stimulus pattern has an evocating and orienting effect on exploratory behaviour and thus imports a structure to it.

Barnett (1958b) has discussed this first group of characteristics. The characteristics of group (2) have been demonstrated in the work of Montgomery and others previously mentioned. Walker *et al.* (1955) have shown that the stimulus pattern is of great importance in inducing spontaneous alternation—a special case of exploratory behaviour.

These experiments were designed to examine the effects of the stimulus components of a situation on the structure of exploratory behaviour. They are thus concerned with two things.

1. The behaviour of the albino rat when first released in an experimental situation it has not experienced before.
2. A method by which this variable behaviour can be quantitatively examined in order to elucidate the relationship between the various types of stimuli present and the structure of the behaviour observed.

The technique adopted took the form of a $2 \times 2 \times 4$ variance analysis of tests performed using one experimental situation. The various stimuli used were distributed in a way which facilitated the analysis of their separate effects. The methods and results are described in chronological sequence in order to prevent unnecessary repetition.

Experimental Methods

1. Pilot Experiment

(a) Would a rat placed on a board sixteen square feet in area explore the entire situation?

(b) Would the rat continue the exploration for long enough for adequate records to be taken of its movements? A ten-minute period was considered adequate for this purpose.

A square of hardboard of side four feet was divided into nine squares each of side sixteen inches, using crayon. The square was set up on the floor of the experimental room of the laboratory at a height of fifteen inches from the floor. In no case during the observations reported here did a rat jump from the square on to the floor during the period of the observation. A rectangular tray twelve inches by nine, spread with clean sawdust was placed in the centre square. The rats were placed on the sawdust at the start of each observation. The sawdust was the normal covering of the floor of their stock cages and provided a contrast with the remainder of the square. The rats were then observed from an adjacent room through an observation panel
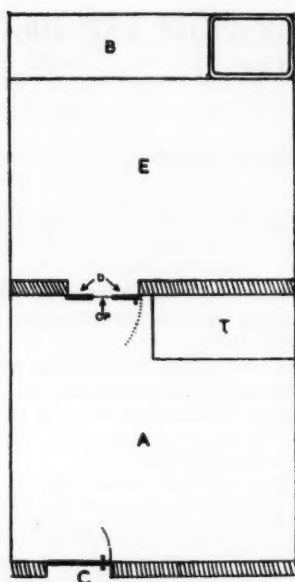
Fig. 1. The Experimental laboratory.
Key: E—Experimental room.
B—Bench with sink, cupboards beneath.
D—Communicating door.
OP—Observation panel.
T—Table with kymograph recorder.
A—Observation room and rat colony.
C—Outer door to corridor.

(Fig. 1) for a period of ten minutes. Each time a rat moved into a square this was noted. This gave the total number of visits, or changes of position, to all nine squares during the observation period. This figure was used as an index of exploratory activity. The rats used were Wistar albino males between the weight range 200-300 gm; one hundred and sixteen in all were used. .

The observation panel used in these tests was suggested by Dr. H. Ascher. It consisted of a sheet of perforated zinc painted white placed in between a sheet of perspex and a sheet of black paper. The paper faced towards the observer and had two eyeholes each two inches in diameter cut in it. The principle employed is that of contrast (Asher, 1950) between the white and black making small variations in the light behind the screen invisible from the perspex side of the screen. The experimental room was illuminated by daylight and the observation room was in darkness. This screen gave a clear view of the behaviour of the rat.

Other work (Chance, 1946; 1956; Sellar & Smart, 1959) has suggested that certain types of disturbance affect the responsiveness of rodents to some physiological tests. Therefore, in this test, five groups with five rats in each group were left undisturbed for five days before the experiment. A further five groups were placed in different, clean cages every day for five days before the experiment. Each day the rats of each group were separated, one to each new cage. Thus no rat remained in the same cage and in the same group for more than twenty-four hours. This procedure will be called social and environmental disturbance.

In view of the work of Hall (1934) on open field situations it was decided to record the number of faecal pellets dropped by the rats during these experiments. This has been interpreted as an index of emotionality. Whilst it is not considered wise to place such broad interpretations on specific aspects of behaviour (Watson, 1958), Hall's work, and that of others, has shown that defaecation and urination are features of the behaviour of exploring rats. Hence, although urination could not be recorded it was of interest to know the effects of the experimental situation on the behaviour of defaecation.

Table I.

Albino male rats 200-300 gm. treatment	Mean number of visits per rat in 10-min. period	Mean number of faecal pellets per rat in 10-min. period
Undisturbed (25 rats)	31·48	4·32
Socially and environmentally disturbed (25 rats)	43·12	2·76

The result of this test is shown in Table I. The differences between the number of visits and between the number of faecal pellets shown are both significant at a P value of 0·05 or less.

All the rats in the test explored for at least ten minutes. Rats which were socially and environmentally disturbed moved about more than rats which had not been disturbed. Rats from socially and environmentally disturbed groups defaecated less than the undisturbed rats.

These findings are discussed later. Their significance at this point in the work was to show that the experimental method permits

quantification of exploratory behaviour in rats whether they were socially and environmentally disturbed or not.

A square of hardboard of side four feet was placed in the experimental room as before. The square was marked out, using crayon, into sixteen equal squares in rows and columns of four as in Figs. 2 and 3.

Fig. 2. The positions in which the rats were placed at the start of the control and experimental tests.

LAYOUT OF EXPERIMENT
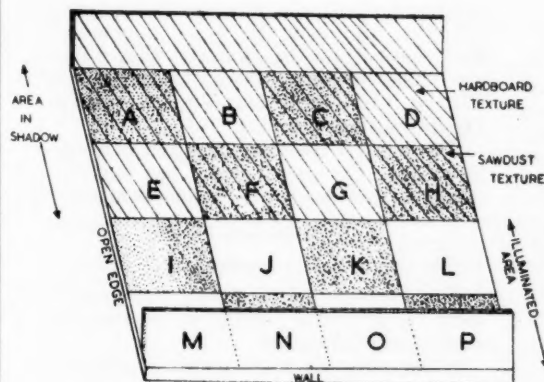
Fig. 3. The experimental design.

A fan was placed on the floor in the right-hand part of the experimental room to control the possibility that the rats would respond to directional olfactory stimuli. The fan was left running for half-an-hour each day before the observations were begun and it was found (using ammonium chloride fumes and fine dust) that a circulation of air was set up which homogenized after twenty minutes. When placed on the square the rat would be subject to olfactory stimulation at first, but it would have no orientating component because of the prior stirring of the air. The possibility that the sound of the fan, or the direction of the slow drift of air from it across the situation, might affect exploratory movements was ruled out by the T test on columns in the two controls. No difference between columns were found.

The rats were kept in groups of four. Each group was placed into a clean cage on the day before they were to be used in the observations. The elaborate procedure of social and environmental disturbance was not considered necessary. The rats used were males between 200 and 300 gm. in weight.

Although sixty-four rats would have given a complete arrangement of starting positions for the rats, it was found that 32 rats yielded sufficiently conclusive experimental data in each test. No further rats were used. In each test the rats were placed on the square in the position shown in Fig. 2.

The movements of the rats were recorded in the following way. Each square was known by a letter and when a rat entered a square the morse symbol for that letter was recorded on the paper of a slowly moving kymograph. A morse key was connected to an electromagnet which altered the position of an ink pen writing on the kymograph paper. The time spent on that square was recorded by the distance between the symbols, which was later measured by means of a calibrated ruler. From these records figures for visits and total time per square were obtained. Consequently, it was possible to produce values and means for visits, time and time per visit for each square over ten minutes for 32 rats.

The illumination of the experimental situation was by means of a sixty-watt pearl bulb which was hung from the ceiling five feet from the square. The bulb holder was fixed inside a box which had light-proof ventilation holes in it. The room was otherwise light-proof. The observations were made between 9.30 a.m. and 6.00 p.m. Throughout the control and the experimental tests the

grid was spread with sawdust after each observation to remove the pools of urine and the sawdust was then removed.

2a. The First Control Test

The first test was performed using the square devoid of the stimuli to be tested to see if the distribution was random. To prevent trail-following at the start of this test thirty rats were released on the square for thirty minutes each day for three days. These rats left pools of urine and faecal pellets all over the grid; there was no special concentration of such pools or pellets in any part of the grid. The first few records were then compared carefully and it was found that they were not noticeably similar or dissimilar.

2b. The Second Control Test

The distribution of readings in the first test was found to be uneven. There were at the time several discontinuities in the room, peripheral to the square. It was thought that the rats may have been reacting to:

1. The observer, who went to the door and closed it after placing the animal on the square (Row 4 being nearest the door).
2. To the door itself, which was recessed about one foot from the wall and was painted brown, the sides of the recess being green.
3. To a tubular light (not used in these experiments) which was found to be not quite centrally placed.

The walls of the room were covered with grey Cellotex to dampen sound transmission and it was thought that perhaps the colour and texture discontinuity of the doorway was influencing the rats. A plank of cellotex was fixed to the door to eliminate this discontinuity. It did not interfere with the observation. The position of the tubular light was also corrected and a second control experiment was performed in the same manner as before except that faecal pellets dropped on the squares were noted in this test.

This test showed that the exploratory behaviour of the rats was being influenced by stimulus discontinuities peripheral to the square and was not affected by the exit of the observer. The result and its effect on the experimental findings are discussed later.

2c. The Experimental Test

The object of the experimental test was to combine a number of stimuli together, in an unfamiliar situation, in a way which would make possible the analysis of the effects of each stimulus on exploratory behaviour.

The unfamiliar situation was composed of the stimuli detailed below as there were indications that these stimuli would affect exploratory movement.

1. Floor texture (a) Sawdust
                  (b) Plain smooth surface (hardboard)
2. Illumination (a) Bright light
                (b) Shadow
3. Barriers to (a) Walls
   movement   (b) Open edges.

It was decided to combine these simple stimuli into a single test situation by constructing a square divided, as previously described, into sixteen squares each one square foot in area. The squares contained the above stimuli in the following way (Fig. 3).

Squares A.C.F.H.I.K.N.P. —Sawdust   Floor texture
        B.D.E.G.J.L.M.O.—Hardboard
Squares A.B.C.D.E.F.G.H.—In shadow } Illumination
        I.J.K.L.M.N.O.P. —In light
Squares A.D.M.P.—Corners (wall open edge)
   „   B.C.N.O. —Walls
   „   E.I.H.L. —Open edges   } Barriers
   „   F.G.J.K. —Open squares
                   (no barriers)

A rectangle the same shape as half the grid was cut out of the lower end of the box over the light bulb and a shade fifteen inches in length was installed to cut down light scatter. The box and the shade were painted matt black to prevent reflection and then the whole apparatus was hung down from the ceiling so that it illuminated half the grid, leaving the other half in shadow. The contrast between the two halves of the grid was quite marked.

The sawdust used was changed between observations. The experimental test was carried out in the same way as the second control test.

Experimental Results

1. The Experimental Readings

The readings taken during the experiment show the following aspects of the structure of exploratory behaviour.

While exploring a rat spends a certain total time at each point in a situation. This time is the result of both the number of visits to a point and the duration of the visits. For example, the same amount of time may be spent at two points. In the one case it might be the result of many brief visits and in the other the result of a few long ones. Therefore, it was necessary to measure

the variance not only of the total time, but also of the number of visits and the duration of visits during the exploration of the situation.

The number of visits to a square is a measure of the tendency for exploratory behaviour to be directed towards the stimuli in the square when the rat is at a distance from it. The duration of the visits is a measure of the tendency for exploration of the stimuli in a square to be prolonged when the rat is on the square.

The arrangement could thus have quantitatively different orientating effects. Exploratory movement (visits), exploratory duration (time/visit) and exploration time (total time) are all aspects of the structure of exploratory behaviour.

The number of faecal pellets voided was also recorded in the second control and the experimental test and in the latter the distribution was noted.

2. The First Control Test

The results are shown in Tables IIA, IIB and IIC. It can be seen that the number of visits was considerably higher in Row 4, the time spent per square higher in Rows 1 and 4 and the time spent per visit was also higher in Rows 1 and 4.

A t test comparison between rows and be-

Table II. (Square indicated by capital letters).
A. 1st CONTROL. TOTAL VISITS.

Row	Total							
1	201	A	47	B	52	C	53	D	49
2	211	E	62	F	42	C	42	H	65
3	243	I	64	J	54	K	53	L	72
4	358	M	73	N	103	O	100	P	82
Total Column		1	246	2	251	3	248	4	268

B. 1st CONTROL TIME (sec.)

Row	Total							
1	3,363·6	A	983·8	B	351·5	C	739·2	D	1,289·1
2	1,443·3	E	441·3	F	249·9	G	160·2	H	591·9
3	2,219·0	I	239·6	J	519·2	K	568·4	L	891·8
4	12,174·1	M	3,788·4	N	1,868·9	O	2,682·1	P	3,834·7
Total Column		1	5,453·1	2	2,989·5	3	4,149·9	4	6,607·5

C. 1st CONTROL TIME/VISIT (nearest sec.)

Row	Total							
1	68	A	21	B	7	C	14	D	26
2	26	E	7	F	6	G	4	H	9
3	37	I	4	J	10	K	11	L	12
4	144	M	52	N	18	O	27	P	47
Total Column		1	84	2	41	3	56	4	94

tween columns showed that differençes for visits and time per visit were significantly affected. Row 4 was visited more often than Rows 1 to 3. More time was spent per visit on the squares in Row 1 than Row 2 and Row 4 than on Rows 2 and 3. The columns did not show any such differences.

The measures taken to counteract this bias in the readings have been described.

3. The Second Control Test

The results of this test are shown in Tables IIIA, IIIB and IIIC. It can be seen that the second control results were biased in the opposite direction.

Again no significant differences were found between any of the measurements in the columns. Row 1 was visited more frequently than Rows 2 and 4. More time was spent per visit on the squares in Row 1 than on Rows 2 and 3.

The only difference between the Control Tests 1 and 2 was the presence of discontinuities—a door in the wall near Row 4 in Test 1, together with the light out of centre. This was corrected in control Test 2, but a cupboard on the wall opposite the door remained to account for the bias towards Row 1 in Test 2.

Apart from the effect of the peripheral situation the construction of the grid itself seemed to be exerting some effect on the measures taken.

Table III.
A. 2nd CONTROL. TOTAL VISITS

Row	Total							
1	366	A	85	B	105	C	98	D	78
2	252	E	74	F	71	G	47	H	60
3	247	I	63	J	55	K	65	L	64
4	226	M	52	N	56	O	64	P	54
Total Column		274 1		287 2		274 3		256 4	

B. 2nd CONTROL. TIME (sec.)

Row	Total							
1	10,453·0	A	3,072·7	B	3,026·5	C	1,664·8	D	2,689·0
2	2,631·3	E	790·8	F	600·8	G	326·0	H	913·7
3	1,976·7	I	315·5	J	244·9	K	752·5	L	663·8
4	4,139·0	M	1,474·6	N	671·8	O	761·3	P	1,231·3
Total Column		5,653·6 1		4,544·0 2		3,504·6 3		5,497·8 4	

C. 2nd CONTROL TIME/VISIT (nearest sec.)

Row	Total							
1	116	A	36	B	29	C	17	D	34
2	41	E	11	F	8	G	7	H	15
3	31	I	5	J	4	K	12	L	10
4	75	M	28	N	12	O	12	P	23
Total Column		80 1		53 2		48 3		82 4	

Table IV. The Result of a Variance Analysis and Orthogonal Comparison of the Values obtained in the Two Control Tests.

Comparison	Source of Variation	First control			Second control		
		Time	Visits	Time/Visits	Time	Visits	Time/Visits
Variance Analysis	Barriers	Significant	N.S.	Significant	Significant	N.S.	Significant
Orthogonal comparisons	No barrier v One barrier	N.S.	N.S.	N.S.	N.S.	N.S.	Significant
	No barrier v Two barriers	N.S.	N.S.	Significant	N.S.	N.S.	Significant
	One barrier v Two barriers	N.S.	N.S.	Significant	N.S.	N.S.	Significant

Significant: Variance significantly affected (5 per cent. level) by the factors.
N.S.: Varairnce not significantly affected by the factors.

The distribution of figures was by no means biased simply in the direction of the discontinuities. The barriers to movement on the grid may have been causing a bias in the readings. Consequently, an analysis of variance of the readings and a set of orthogonal comparisons were made (see Section 4).

On the grid there were four centre squares with no barriers, eight peripheral squares with one open edge and four corner squares with two open edges. The results of this analysis are summarized in Table IV.

In both the control experiments it was found that the barriers were affecting the time spent per visit, and, therefore, in the variance analysis only, the total time. This was probably due to the physical effect of the barriers to movement. The rat comes to the open edge, stops and turns round before moving away. This process would be most time consuming at the corners, less so at the edges and least in the centre squares and this is the way in which the figures were biased. The open edges exerted no effect on the number of visits.

The number of faecal pellets voided by each rat was recorded. The mean was found to be 3·5 per rat. No record was made of the distribution of the faeces.

4. The Experimental Test

The control experiments showed that investigatory movements could be influenced by the stimuli in the experimental situation. It had been thought that the experiment should be delayed whilst the method of securing a random distribution for a control test was found. However, it was impossible to overcome the effects of all such peripheral discontinuities in the experimental room and it was not known whether the actual experiment would yield positive results. The experiment was, therefore, performed without further alterations to the room.

The experimental results are summarized in Tables V_A, V_B and V_C. These figures were subjected to a $2 \times 2 \times 4$ variance analysis and it was found that the variance ratio for the interactions between factors were all non-significant. Combining these interactions into a pooled error it was found that there were, however, significant differences (5 per cent. level) in the distribution of some of the measurements.

As before, the variance analysis did not give enough information about the nature of the effects of the different types of barriers. Accordingly the figures were combined in the following orthogonal comparisons.

Orthogonality is a property of experimental material which ensures that the different classes of effects to which experimental material is subject shall be capable of direct and separate estimation without any kind of entanglement. It can be seen that different groups of squares can be selected which duplicate for all factors except comparisons between the effects of barriers. Accordingly, such selections were made for the following orthogonal comparisons:

1. A comparison between the effects of walls and open edges on the periphery of the square, including the corner squares.
2. A comparison between the effects of the

Table V.

A. EXPERIMENTAL. TOTAL VISITS.

Row	Total				
1	796	A 155	B 266	C 240	D 135
2	398	E 97	F 110	G 92	H 99
3	299	I 68	J 64	K 50	L 73
4	362	M 72	N 99	O 110	P 81
Total Column		392 1	539 2	492 3	388 4

B. EXPERIMENTAL. TIME (sec.)

Row	Total				
1	13,032·6	A 4,351·5	B 2,875·5	C 4,235·7	D 1,569·9
2	2,114·7	E 671·4	F 576·1	G 288·8	H 578·4
3	1,428·0	I 672·4	J 314·7	K 162·8	L 278·1
4	2,624·7	M 652·4	N 378·3	O 441·3	P 1,152·7
Total Column		6,347·7 1	4,144·6 2	5,128·6 3	3,579·1 4

C. EXPERIMENTAL. TIME/VISIT (nearest sec.).

Row	Total				
1	69	A 28	B 11	C 18	D 12
2	21	E 7	F 5	G 3	H 6
3	22	I 10	J 5	K 3	L 4
4	31	M 9	N 4	O 4	P 14
Total Column		54 1	25 2	28 3	38 4

barrier content of corner squares and no barriers as in the centre squares.

3. A comparison between the overall effects of barriers when associated together (corners, centre quares) with their effect when each is separately represented (peripheral squares not including corner squares).

The results of these comparisons can be summarized in Table VI and are as follows:

(a) Differences due to texture are not significant.

(b) The squares in shadow are visited more frequently than those in the illuminated area. This is presumably the cause of the greater amount of time spent in the area in shadow,

since the time spent per visit was unaffected by illumination.

(c) The barriers affect the number of visits and the time spent per visit for the squares in the same way; the time measure is not affected.

(d) The orthogonal comparisons show that walls are visited significantly more often than the open edges. The time spent per visit and consequently the total time are both significantly greater for the corner squares than for squares with no barriers. The number of visits was greater to the eight squares containing walls and edges separately than to the eight containing the same number and type of

Table VI. The Results of the Variance Analysis of the Experimental Values and Consequent Orthogonal Comparisons.

Comparison	Source of variation	Measurement		
		Total time	Total visits	Time/visit
Variance analysis	Texture	N.S.	N.S.	N.S.
	Illumination	Significant shadow greater	Significant Shadow greater	N.S.
	Barriers	N.S.	Significant	Significant
Orthogonal comparisons	Walls and open edges	N.S.	Significant walls greater	N.S.
	Open edges and corners	Significant corners greater	N.S.	Significant corners greater
	Open squares and corners v. walls and open edges	N.S.	Significant. Walls and edges greater	N.S.

N.S.: Variance not significantly affected by the factors.
Significant: Variance significantly affected by the factors.
Example: Significant. Walls greater—variance significantly affected in the visit values.
     Walls are visited more often than open edges.)

barriers associated in the form of corners in four squares. This did not significantly affect the total time measure.

The mean number of visits per square in the two control tests were not significantly different (first control 63·3, second control 68·2). Both were significantly different from that found in the experimental test (113·2). The number of visits in the experimental test was greater per square in the area in shadow. A comparison of the mean number of visits per square in the illuminated half of the experimental test (77·125) and those of the two controls showed that they were not significantly different. The difference between the control and experimental tests in this respect was due to the experimental differential illumination.

The mean time spent per visit in the two controls were similarly found not to be different (first control 17·2 seconds, second control 16·4 seconds). The mean for the experimental test (8·9 seconds), differs significantly from them both. This measure was not affected by illumination in the experimental test.

The mean defaecations per rat in the second control (3·5) are significantly different from that in the experimental test (4·8). No measure of defaecation was recorded in the first control. An analysis of the variance of the distribution of faeces was made and the result is summarized in Table VII. This showed that illumination differences and barriers affected the distribution

of the faecal pellets and that textures did not. This corresponds with the variance of the number of visits. Consequently no orthogonal comparisons were made in this case.

Table VII. The Result of an Analysis of the Variance of Faecal Distribution in the Experimental Test.

Source of variation	Distribution of faeces
Texture	N.S.
Illumination	Significant
Barriers	Significant

Significant: Variance significantly affected (5% level) by the factors.
N.S.: Variance not significantly affected by the factors.

5. A Critical Examination of the Result

There are some criticisms which can be made about the validity of the experimental results. First, the experiment was performed in a peripheral situation which was known to exert effects on the measures taken in a similar way to those found in the experiment. Second, the differences found in the experiment due to barriers may have been due to the physical effects found in the two control experiments. In other words, some of the experimental results may not be valid.

In the second control test the number of visits

to Row 1 were greater than to any other row and the other rows did not differ from each other in this respect. Row 1 was nearest to the cupboards which presented a discontinuity in the peripheral environment to which the rats were apparently reacting. A row and column analysis of the experimental test for visits shows that this situation is not the same, but is similar. Row 1 was visited more frequently than Rows 2, 3 and 4 in the same manner as in the control. The column comparisons were again not significant. Therefore, a possible objection could be raised that the experimental distribution was not due to the stimuli on the square despite the fact that stimuli peripheral to the square were in semi-darkness.

If this were so the walls and the shadow would not have influenced the distribution of visits to other rows, but this was found to be the case.

(a) The visits to Row 4 (wall) were greater than those to Row 3 (no wall); both these rows were in the illuminated area.
(b) The visits to Row 2 (shade) were greater than those to Row 3 (light); both these groups had no walls.
(c) The visit to Row 1 (wall) were greater than those to Row 2 (no wall); both these rows were in the non-illuminated area.
(d) The visits to Row 1 (shade) were greater than those to Row 4 (light); both these rows had walls.

A series can be prepared to illustrate these effects further:

Row 3 (no wall or shade)—Mean visits—63·75 ⎫ not sig-
Row 4 (wall, no shade)   ,,   ,,   —90·5 ⎬ nificantly
Row 2 (shadow, no wall)   — ,,   ,,   —99·5 ⎪ different*
Row 1 (shadow and wall)   — ,,   ,,   —199 ⎭

*All other differences insignificant.

These results support the view that illumination differences and the walls exerted the effects on exploratory movements as shown in the analysis of variance.

The possibility remained that the physical effect of the barriers to movement may have been imparting a spurious significance to the effect of walls on the movements of the rat. This view was supported by the fact that the time per visit was affected in the same way by barriers in the controls and the experiment. However, it was found that the barriers contributed significantly to the variance of the visits in the experimental test. This was not so in the second control test (Table IV). The experimental orthogonal comparisons showed that walls are visited significantly more often than the open edges. Therefore, it is concluded that the presence of walls influenced the distribution of visits.

There is no evidence that the corners between walls and open edges exerted any marked effect on exploratory behaviour. It appeared that they

were visited less often than squares containing the same content of barriers not associated together (see Table VI).

The fact that there were no differences due to the floor texture was rather surprising. Differences due to texture were analysed separately in the light and dark halves of the situation and were found not to be significant. Each row was duplicated for all factors, but texture. The differences due to texture in the single rows were separately examined and were also found to be not significant.

Further evidence that the experimental test exerted different effects on the behaviour of the rats was found. The mean time spent per visit, the mean number of visits and the mean defaecations per rat were significantly different between the experimental test and the control tests.

In view of these findings it was decided that the effects shown in the variance analysis of the results of the experimental test were real. The method is thus valid as an experimental procedure for certain types of behaviour problem.

Discussion

In previous work (Chance & Mead, 1955) the behaviour of rats in new or changed surroundings was described as investigatory. In view of the scope of the present application of the term exploratory behaviour, the behaviour reported here has been described as exploratory pending further discussion of the interpretation of variable behaviour.

The experimental test showed that exploring rats move about more (visit more) in shadow than in light and that they move in relation to walls more than open edges. Neither of these results is surprising. It was, however, expected that the floor texture differences would have an effect. The rats had lived on sawdust floors throughout their lives. They had not previously experienced a smooth hard texture like the alternative. There was a very little spread of sawdust on to the other squares during the observations. No effect of floor texture was found. The results show that the total time spent exploring part of an experimental situation is a measure of limited value. Table VI demonstrates that the variance of *either* the number *or* the duration of visits can significantly affect the total time measure.

The features of exploratory behaviour examined here have been those of change of position (visits), duration of position (time/visit) and defaecation. It has been shown that the components of the stimulus pattern of a completely

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new situation have different effects on these features. To that extent such stimulus components may be interpreted as having an orientating effect on exploratory behaviour. Exploratory behaviour is also affected by previous treatment unrelated to the test situation.

The treatment of social and environmental disturbance produces more exploratory movement and less defaecation than is shown by rats not so disturbed.

Some workers (Montgomery, 1954; Zimbardo & Montgomery, 1957) have referred to exploratory behaviour or a drive. Barnett (1958b) does not commit himself on this point. If the activity of exploration has the characteristics of a drive it is to be expected that continuous change for five days, evoking exploration each day, would exhaust the drive to some extent. In contrast, the undisturbed rats would be expected to have an unexhausted exploratory drive on the day of the test. Theoretically the test situation should present a contrast of the same magnitude to both groups of rats. On these premises the undisturbed rats should show exploratory behaviour of a greater intensity than that of the disturbed ones. However, the disturbed rats move about the situation more while exploring than the undisturbed ones. The fact that the rats changed position more frequently indicates a greater level of exploratory activity. The question of exploratory intensity is discussed later. However, drives are regarded as being *internal* states inducing behavioural activity which is said to be motivated when it becomes specifically directed towards some incentive (Munn, 1950). In this case, the additional activity is significantly related to prolonged changes in the external environment. The effects expected of a state of endogenous activation do not occur. As Barnett remarks: "Exploration, therefore, obviously demands a theory of motivation more subtle and complex than those which have prevailed hitherto." (1958b, p. 305).

Exploratory behaviour is not merely an expression of a general tendency to be active. This has been demonstrated by Berlynne (1950) and Montgomery (1952b; 1953). This does not eliminate the possibility that specific stimuli exert a kinetic effect on exploratory behaviour which would ultimately tend to terminate it. If this is so then visit duration would be expected to increase with a decrease in visit number. This did not occur in this experiment.

The duration of visits in the varied stimulus situation was less than that in the plain one. In contrast, the exploratory movement was significantly increased only in the area in shadow in the experiment. This effect is different from that caused by social and environmental disturbance which caused an overall increase in movement. The bearing of these results on the assessment of the intensity of exploratory behaviour will now be considered.

All the available evidence indicates a direct, but by no means simple, relationship between exploratory behaviour and external change. A greater degree of external change produces the effect of greater intensity in exploratory behaviour when measured in terms of a different, simultaneously evoked response (Chance & Mead, 1955). They also indicated that the magnitude of the effects of external changes varied with time. It is possible that the intensity of exploratory behaviour is shown in terms of the amount of movement and in duration of position. A more varied situation may produce a greater effect than a plain one; here the duration of visits decreased in the varied situation. Previous disturbance may produce a greater degree of activation than no disturbance; here the visit number increased in the disturbed rats. No conclusion is possible from these data but further work may validate this hypothesis.

The visit number and visit duration measures are to a great extent mutually related. However, the results of this work show that the conclusions which can be drawn from this relationship are not always true. For example, a comparison between the second control test and the experiment shows that visit numbers was not significantly affected in the illuminated part of the varied situation, whereas duration of visits is affected.

The behaviour of defaecation while exploring might have been having the effect of prolongation on the duration of the visits. General observations on rats show that they defaecate both when they are moving and immobile. The defaecation increase in the varied situation was accompanied by a decrease in the duration of the visits. Moreover, stimuli which affected the variance of the faecal distribution were the same as those affecting visit number. Duration of visits were not affected in the same way.

Exploring rats defaecate more in varied surroundings than do those in uniform situations. The external environment thus has an influence on defaecation. Undisturbed rats defaecate more while exploring than do rats which have been socially and environmentally disturbed. Defaeca-

tion can thus be affected by previous disturbance. In these observations the changes in amount of defaecation were general. In contrast in the work of Hall (1934) such increases in defaecation were observed in "emotional" rats. The relationship between the behaviour of defaecation and the factors evoking exploratory behaviour is not clear. It has not yet been considered whether defaecation is an essential feature of exploration. It is, therefore, suggested that defaecation be noted in future work on exploratory behaviour.

In conclusion it must be emphasized that these results, and consequent suggestions, apply only to the initial exploratory behaviour of rats. A brief ten-minute period of observation will not yield results conclusively applicable to any aspect of prolonged exploratory behaviour. This is a separate problem, not considered here.

The main contribution arising from this work has been the development of a technique for the examination of the effect of different stimuli in a test situation on some aspects of the structure of exploratory behaviour. Further work on these lines might yield valuable information about the stimuli evoking and directing exploratory movement. The method could also be adapted for tests on sensory discrimination. If exploratory behaviour is modified by the presence, absence and distribution of a particular kind of stimulus it follows that it can be perceived by the rat.

Summary

1. A method is described for the investigation of the effect of sensory data on the structure of initial short-term exploratory behaviour. It is suggested that this method could be modified for use in sensory discrimination tests.

2. Rats which have been socially and environmentally disturbed change position more, when exploring a new situation, than do rats which have not been so disturbed.

3. Social and environmental disturbance before the exploration of a new situation causes a decrease in the amount of defaecation during tests.

4. The structure of exploratory behaviour of rats on a plain square test situation is affected both by the physical structure of the square and by stimuli peripheral to it.

5. Floor texture differences between hardboard and sawdust do not affect initial exploratory behaviour.

6. More exploratory movement occurs in an area in shadow than in an area brightly illuminated. This is not regarded as a kinetic effect. There is no difference of visit duration between shaded and illuminated squares.

7. More exploratory movement takes place in relation to walls than in relation to open edges. There is no difference between the time spent at any point along either of these types of barrier.

8. Rats change position more and remain in one place for less time each visit when in an unfamiliar situation composed of different types of stimuli than is found in the exploration of a more uniform situation. The number of visits is increased in the experimental test in relation to illumination differences. The mean number of visits is high in the area in shadow. The number of visits per square in the illuminated part of the varied situation is not different from that in the uniform square. The duration of visits to the squares in the varied situation is not affected by illumination differences.

9. Variety of stimuli in an unfamiliar situation causes a greater amount of defaecation on the part of rats exploring the situation than is found in uniform situations.

10. Illumination differences and barriers to movement affect the distribution of faecal pellets dropped by exploring rats. Exploratory movement is affected in the same way but the time spent at any point is not.

11. The significance of these results to considerations of exploratory drive, exploratory intensity and the relationship between defaecation and duration of position are discussed.

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RANGING BEHAVIOUR STUDIES WITH ROMNEY MARSH AND CHEVIOT SHEEP IN NEW ZEALAND

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Introduction

This project is one of a series designed to establish physiological characteristics which may be used to distinguish and explain differences between Romney Marsh and Cheviot sheep in relation to their comparative suitability for hill farms in New Zealand. Ranging behaviour studies were included because the ancestral backgrounds and general assessment of the breeds indicated that the Cheviot should be very much the more active of the breeds and perhaps better able to deal with hill country. Therefore the aim of this investigation was to compare the mileages travelled by Romney Marsh and Cheviot sheep grazing on hill and flat lowland pastures in New Zealand. It was also hoped that it would be possible to study (a) the effect on activity of different pastures within the above two locations and (b) the result of switching the two breeds from hill to flat lowland and from flat lowland to hill pastures.

Literature dealing specifically with the distances travelled by grazing sheep is very scanty but indicates breed differences in pattern of behaviour and activity. Louw *et al.* (1948) used a checker-board technique in which they divided pastures into 20-acre squares by means of stakes. Hourly observations made by means of binoculars traced the movements of sheep breeds in relation to the stakes. It was concluded that of the four breeds and crosses investigated on an activity basis the Blackhead Persian could be grouped with the Dorset × Persian and likewise the Merino could be grouped with the Karakul. The two breeds in the former group were more active than the two in the latter. All sheep walked more in summer than in winter probably on account of the longer days in summer. Tribe (1949) studied the general features of the grazing behaviour of Cheviot sheep on one acre of lowland pasture over continuous periods of 24 hours during 12 consecutive months. The one acre pasture was divided by stakes at intervals of ten yards following the method of Wallace & Kennedy (1944). He reports that the

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sheep walked an average of 2·6 miles per 24 hours; 2·06 miles being between 7.00 a.m. and 7.00 p.m. and 0·55 miles between 7.00 p.m. and 7.00 a.m. Most travelling was done in the spring and autumn, and least in summer. The animals walked proportionately more at night in summer than in winter. (Tribe does not mention the possible relationship between the short night of the northern summer and these figures). The method adopted was to follow the behaviour of only one sheep at a time. Tribe reports that in confirmation of the work of Stapledon & Jones (1926) and Jones (1928), it was found that the records from a single animal, on each occasion reflected the actions of the experimental group.

England (1954), using an observation technique for two separate 24-hour periods, studied four ewes of different breeds together with their lambs on two plots each of 1·9 acres. He found that the sheep increased their per diem travel on a bare pasture as compared with one offering abundant herbage as follows: Spanish 2,400-3,876 yd.; Clun 2,268-3,772 yd.; Suffolk 1,922-4,248 yd. and Scotch Blackface 1,558-2,888 yd. On the lush pasture the sheep could be ranked as above for activity but on the bare pasture the Suffolk took the lead the others remaining in the same order. On neither pasture did the sheep travel between midnight and 5 a.m. Although he gives no distances, Hunter (1954), observes that hill sheep are more active than lowland sheep during the hours of daylight. No data were available on the night behaviour of hill sheep but he believed it quite probable that they are more active than lowland sheep over 24-hour period. Unfortunately, in his arguments Hunter appears to overlook the fact that he compares not types of sheep but locations. In a one-year comparative study of the grazing habits of sheep carried out by Van Rensburg (1956) four breeds and crosses of sheep were studied; the technique is not specified. The breeds could be ranked in descending order for daily distance travelled as follows: German Merino × Merino Cross, Dorper, Blackhead Persian, Merino.

Tribe (Hammond, 1955) reviews the literature on grazing behaviour studies. He con-

A. Rangemeter and harness.
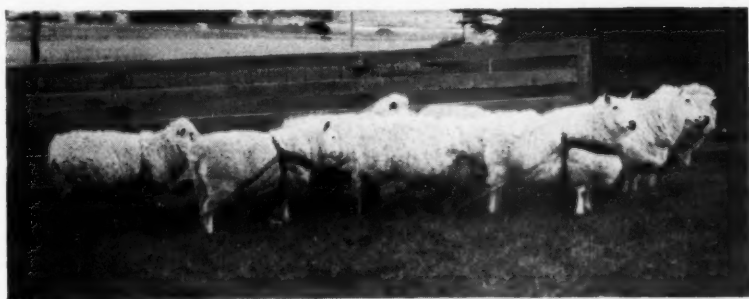
B. Rangemeter sheep in a pen.

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siders that the differences between the animal behaviour patterns described by various workers are not surprising. In addition to the differences in observational technique, there is a complex of environmental factors which must be expected to influence critically a normal pattern of behaviour. For example such variable factors as the climate, the density and quality of the sward, the size of paddock, the system of grazing management and of course the individuality of the grazing animal may influence very significantly the times devoted to different activities.

Materials and Methods

For this project, the problem of measuring sheep activity as expressed in miles travelled was considered from the following angles.

1. It was necessary to measure the ranging mileage of the sheep under natural conditions on hill and lowland grazings. For this reason, it was impossible to use the checker-board technique (Wallace & Kennedy, 1944). Even where the terrain would permit subdivision, no time was available for 24-hour observation shifts.

2. Radio control, pedometers and other such devices for tracking movement were all too complicated or impossible to use satisfactorily on the grazing sheep.

The restrictions thus imposed on the work subsequently led to the development of an entirely new device called a Rangemeter (Cresswell, 1957). The rangemeter (Plate IA) consists of a light-weight harness, two metal shafts and a chassis which, sprung onto the ground, carries a small land wheel which motivates an automatic mileage recorder—a Lucas cyclometer*—as used on bicycles. The total weight of the machine was about eight pounds, but the sheep only carried one pound or half the "spring loaded" weight of the shafts which at one end are pivoted on the shoulder harness and at the other skidded on the ground. The draught of the machine was exceedingly light, a pull of only approximately one pound being required to move it along the flat and on the typical hill slope.

Development of the first machine took place at Massey Agricultural College using one Romney ewe. Further machines were constructed as soon as it was obvious that the principle was sound and initial mechanical defects were remediable. There was no tendency for wheel slip to occur. As can be seen from Plate I, the pivoting of the wheel chassis means that its whole weight

*Joseph Lucas & Co. Ltd., Birmingham, England.

bears down onto the ground. In addition, the pivot is fitted with a spring which allows the wheel to rise and fall with the terrain but nevertheless keeps it firmly pressed onto the ground. This spring is essential to the success of these machines. The original harness was quite elaborate but the final and most satisfactory type developed was that shown in Plate IA†. The machines complete with harness could be removed from, or replaced on, the sheep in a matter of seconds merely by manipulating one buckle. This simplicity of arrangement was beyond value to the success of the work. It is estimated that some of these machines must by now have travelled approximately 500 miles which is a testimony to their design and construction.

On 17th July 1956, rangemeters were attached to five each of mature pregnant Romney and Cheviot ewes selected from similar hill pasturage and with a New Zealand hill environment ancestry. The sheep became accustomed to being in harness surprisingly quickly for after an hour or so they were grazing normally and were then left over night. The following morning the sheep were turned onto an 18-acre hill paddock. This paddock was extremely steep and rough, contained a central ridge and a small stream and was well littered with stumps, logs, old fencing posts, etc. The height of the central ridge was about 500 feet and the gradient from the sheep pens at its base direct to the top in places approached one-in-one. No better testing ground could have been found for both machines and sheep. Although of a precipitous nature the hill carried an abundance of grazing throughout the year. The rangemeter in no way hindered the movements of the sheep which walked, galloped, lay down and got up quite normally. Only twice during the whole course of the investigations did a sheep become hung up on a log or fence post. Experimental animals were accepted by the other sheep who evinced only curiosity about their changed sisters. After parturition the ewes were quite able to suckle their lambs whilst in harness (Cresswell, 1957).

Recording commenced on 28th July, 1956, and from then until August, 1957, the machines were removed only for repairs, lambing, tupping, shearing, and foot dressing. It was soon discovered that once a sheep was broken to harness it never forgot and could be left for weeks between work periods without it becoming excited when harnessed up again. Mileage records

†An improved rangemeter is to be reported on in a later communication.

were read every Saturday morning starting at
8.00 a.m. when the sheep were mustered from
the same night camping spot to a nearby yard or
field pen (Plate IB). Thus gathering errors were
reduced to a minimum.

During the whole period of the rangemeter
project the ewes were run as normal sheep. The
sheep stayed in good health, this being amply
borne out by the weights of the ewes on the
24th August, 1957 (Table I). It will be seen that
both Cheviots and Romneys were above

**Table I. Comparison of Haematology Flock* and Range-
meter Sheep Weights.**

	Haematology flock (30/7/57)			Rangemeter sheep (24/8/57)		
	lb. wt.	S.D.	n	lb.wt.	S.D.	n
Cheviots	125·4	10·9	35	134·4	10·0	5
Romneys	120·1	11·6	35	141·0	16·7	4

*A flock of sheep created for haematological studies.

the mean figures established for other ewes run-
ning on similar country (approximately four
weeks earlier). Some of this extra weight was no
doubt due to the growth of the foetus but even
allowing for this the sheep were in very good
condition.

As a part of the investigation it was decided
to measure the foreleg lengths of the rangemeter
sheep and 35 ewes of each breed maintained in
another flock of comparable animals for
haematology studies. The measurements taken
were height at withers, height from ground to
brisket and the distance from the point of the
elbow to the coronet. Measurements were taken
with sliding calipers and a flexible linen tape.
All measurements were taken four times to the
nearest half inch, the sheep being moved and re-
settled in a normal standing position between
the measurements (Table II).

Results

The mean weekly mileage records and the
weather and pasture data presented in Table III
demonstrate:

1. In general, on the hill the Cheviot ranged
about 8·0 miles per week and the Romney about
5·1 miles per week. On flat lowland the Cheviot
covered about 9·8 miles per week and the
Romney about 8·1. In eight periods out of eleven
(Table IV) the difference between the breed
means was statistically significant.

**Table II. Comparison of Foreleg Measurements,
Haematology and Rangemeter Sheep.**

	Cheviots			
	Haematology flock		Rangemeter shee~	
Height at withers	22·5"	0·9"	23·0"	0·6"
Height to brisket	11·4"	0·9"	11·9"	0·8"
Elbow to coronet	13·3"	0·5"	13·4"	0·2"
	Romneys			
Height at withers	23·2"	0·9"	23·6"	0·8"
Height to brisket	11·9"	0·8"	12·5"	0·7"
Elbow to coronet	13·5"	0·6"	13·3"	0·3"

2. Within the same paddock the breeds raised
or lowered their mileages together as in Periods
A & F.

3. The Romneys raised their mileages when
transferred from hill to flat lowland and then
dropped them sharply when returned to the hill.
For example in the change from the 18-acre hill
to the 6-acre flat paddock (Periods A-B) the
Romneys raised their mileage from 4·4-7·8
miles while, after an initial check which is dis-
cussed later, the Cheviots raised their mileage
only from a hill mileage of 8·2 to a flat lowland
mileage of 8·8. In the transference from flat
lowland to hill the Romneys fell from 6·8-4·1
miles while the Cheviots actually raised their
level of activity. The changes in activity between
different periods were statistically significant in
7 cases out of ten for the Romneys and in only
2 cases out of ten for the Cheviots (Table V).

4. The levels of activity of the breeds were
approximately the same on 27th July, 1957, as
they were one year later on 4th August, 1956.

5. There was a steep rise in the activity of both
breeds before tupping (Period E) and the high
level of activity was repeated on the one week
recorded after tupping before the sheep were
returned to the hill. The differences between
weeks in period E were statistically significant
(Table IV).

6. Identical or almost identical breed mile-
ages occurred very frequently for consecutive
weeks in the same pasture (This was most strik-
ing for individual sheep).

7. Weekly climate could not be related to
distance travelled except in the case of the
Cheviots in a wet week shortly after shearing.
(This week 24th November-1st December, 1956.

Table III.

Dates	Mean weekly mileages			Size and location of pastures	Weekly				
	Cheviots	Romneys	Period		Number of rainy days	Inches of rainfall	Mean minimum temp. °F	Mean maximum temp. °F	Mean wind miles per day
1956									
28 July—4 Aug.	8·9	4·9	}X	18 acre hill	3	0·42	40·0	54·1	93·7
4-11 Aug.	9·0	4·4			5	0·38	33·9	50·8	123·5
Machines removed for lambing									
22-29 Sept.	9·0	4·4			2	0·60	39·9	66·7	92·0
29 Sept.-6 Oct.	7·2	4·0			7	2·05	48·8	61·4	113·5
6-13 Oct.	8·7	5·1	A	18 acre hill	3	0·24	45·6	60·0	114·4
13-20 Oct.	6·8	4·6			7	0·14	47·3	64·3	106·3
20-27 Oct.	6·3	4·0			2	0·78	47·6	66·6	86·3
27 Oct.-3 Nov.	6·3	4·0			6	0·90	54·3	70·1	105·7
Machines removed for shearing									
10-17 Nov.	3·7	1·9			4	0·85	46·8	65·6	89·3
17-24 Nov.	8·2	4·4			3	0·31	54·3	72·0	65·2
24 Nov.-1 Dec.	4·9	7·8			5	0·54	44·4	58·3	112·2
1-8 Dec.	8·8	7·5			2	0·32	48·4	68·6	79·2
8-15 Dec.	9·6	9·1	B	6 acre flat	3	2·33	54·2	68·4	86·6
15-22 Dec.	10·3	8·8			3	1·20	54·4	69·7	114·0
22-29 Dec.	7·0	6·8			4	2·19	54·1	69·4	89·3
29 Dec. 1956- 5 Jan. 1957	7·8	4·1			6	1·76	56·0	68·1	133·9
5-12 Jan.	7·9	4·5	C	18 acre hill	3	1·66	54·8	72·0	105·7
12-19 Jan.	7·9	5·2			0	0·00	55·4	75·3	113·7
Machines removed for repairs									
9-16 Feb.	7·8	6·6			0	0·00	52·2	78·1	108·3
16-23 Feb.	7·7	5·9	D	81 acre hill	3	0·58	57·9	76·0	107·3
23 Feb.-2 Mar.	7·8	5·6			2	0·83	52·9	74·9	99·2
2-9 Mar.	8·7	6·5			1	0·01	56·1	76·6	55·2
9-16 Mar.	10·9	7·6	E	3 acre flat	5	0·80	61·6	75·6	156·6
16-23 Mar.	11·3	10·0			4	1·66	55·0	78·4	118·2
Machines removed for breeding time									
10-27 Apr.	13·9	10·6			7	3·03	52·0	65·9	67·4
27 Apr.-4 May	7·9	3·8			3	0·22	51·1	68·4	69·2
4-11 May	11·6	7·8	F	18 acre hill	5	0·84	44·7	62·5	84·9
11-18 May	8·4	4·6			6	2·66	46·6	60·0	114·0
18-25 May	7·8	4·8			7	2·03	45·5	56·2	95·0
25 May-1 June	9·1	7·5	G	1 acre flat	6	0·53	41·2	55·8	103·2
1-8 June	10·9	8·1			4	0·35	35·1	54·0	83·9
8-15 June	7·8	4·7	H	18 acre hill	5	0·86	42·7	56·7	115·0
15-22 June	8·0	4·5			4	0·83	43·2	56·5	114·0
Machines removed for foot-dressing of sheep									
29 June-6 July	9·7	5·9	I	49 acre hill	6	1·77	46·1	54·8	126·3
6-13 July	11·8	5·9			0	0·00	29·9	50·7	39·9
13-20 July	9·3	5·5			4	0·50	36·1	55·9	90·6
20-27 July	9·4	5·1			5	0·97	38·0	53·4	96·7
27 July- 3 Aug.	8·3	5·9	J	18 acre hill	3	0·11	33·3	51·8	104·0
3-10 Aug.	9·3	8·0			2	0·40	48·8	60·2	197·8
10-17 Aug.	9·4	6·9			3	0·40	45·8	59·1	100·4
17-24 Aug.	9·7	7·9			4	0·46	41·5	56·4	123·6

Table IV. 1. Average % Greater Weekly Mileage Travelled by Cheviot over Romney Sheep, within Period.* 2. Significance of difference in Weekly Mileages Between Breeds within Periods using Week Means. 3. Significance of Difference Between Weeks within Periods within Breeds.

Period	Location	Average % greater mileage in favour of Cheviots	Level of significance of difference between breeds	Significance of difference between weeks within periods within breeds	
				R	C
X	18 acre hill ⎤		1%	1%	
A	18 acre hill ⎦	69%	1%		
B	6 acre flat	29%	no significant difference		
C	18 acre hill ⎤		1%		
D	18 acre hill ⎦	51%	5%		
E	3 acre flat	30%	5%	5%	5%
F	18 acre hill	75%	approaches 5% level†		
G	1 acre flat	27%	no significant difference		
H	18 acre hill	72%	5%		
I	49 acre hill	82%	5%		
J	18 acre hill	45%	1%		

*For the identification of the periods see Table III.
†Highvariation between weeks within breeds.

Table V. Levels of Significance of Difference in Weekly Mileages Between Consecutive Periods within Breeds Using Sheep Means.

Location	Periods*	Cheviots	Romneys
18 acre hill—18 acre hill	X — A		
18 acre hill— 6 acre flat	A — B		1%
6 acre flat—18 acre hill	B — C		1%
18 acre hill—18 acre hill	C — D		5%
18 acre hill—3 acre flat	D — E	1%	1%
3 acre flat—18 acre hill	E — F	approaches 5%	5%
18 acre hill—1 acre flat	F — G		1%
1 acre flat—18 acre hill	G — H		1%
18 acre hill—49 acre hill	H — I	5%	
49 acre hill—18 acre flat	I — J		

*For the identification of the periods see Table III.

was the only time at which Cheviot mileage fell below the Romneys. It is interesting to speculate whether or not a possible similar reaction to bad weather on the part of the newly shorn Romneys was negated by their favourable reaction to lowland pasturage.

8. Variations in the size of flat lowland paddocks did not result in corresponding regular

variations in the distances travelled by both breeds. A large increase in the acreage of hill pasture resulted in both breeds increasing their mileage by about one third.

9. Lactation did not result in increased mileages although they did rise during the latter stages of pregnancy in 1957.

10. Wide weekly and seasonal variations in quantity of pasture on the same paddock did not affect the distance travelled except perhaps during pregnancy in 1957.

Analysis of the leg measurement records (Table II) showed that the Romneys were approximately $\frac{2}{3}$ inch greater in height at the withers, $\frac{1}{2}$ inch greater in height to the brisket and $\frac{1}{10}$-$\frac{3}{10}$ inch greater in elbow coronet measurement.

Discussion

The records reported show that of the animals under investigation the Cheviot, the shorter legged breed, was 50 to 100 per cent. more active than the Romney under hill conditions. Under lowland conditions, however, the Romney increased its distance travelled and, in fact, very nearly equalled the Cheviot, only to fall back again quite decisively when returned to the hill. The fact that the Romney greatly increases its activity on flat lowland pastures contradicts the commonly held idea that the Romney Marsh sheep in New Zealand has adapted itself to hill grazing.

Except for on the hill no regular support was found for those theories (Hammond, 1955) which would associate larger and/or sparser pastures, and also lactation, with greater grazing distances. Neither was it possible to reconcile the results with the writings on weather effects (snow was not included) and it is felt that others may have overlooked the fact that for all intents and purposes the sheep carries its own environment around with it in the form of its fleece. The rise in the activity of the pregnant animals contradicts the shepherds' traditional idea that in-lamb ewes spend more time lying down as parturition approaches. However, the frequency with which sheep put up the same mileage in the same pasture supports the belief of many shepherds that sheep establish a home within a paddock (Firbank, 1940). This could also help to explain the working of hefted sheep on unfenced hills in Great Britain. The increase in activity during the breeding season is of notable interest for at that time it is possible that sheep are naturally metabolizing at a low rate (Cress-

well, 1958)—a contradiction which it is hoped to investigate further.

The greater leg length of the Romney was of course to be expected for the Cheviot is generally speaking the smaller of the two breeds. Although the measurements do not agree with those of von Borstel (1951) taken on ten animals of each breed (he found unshorn Cheviots to be about one quarter of an inch higher at the withers than unshorn Romneys and one twentieth of an inch higher when shorn), it would appear that the greater distances travelled by the Cheviots cannot be related to a greater leg length. This is of some immediate interest for it has been inferred that longer legs will impart to the Romney greater ease of travel and better leverage for hill climbing (Barton, 1954).

It is recognized that these results were obtained in a New Zealand environment and are thus not strictly comparable with, say, work in South Africa or in the North of Scotland, Nevertheless, it is suggested that further enquiry on the lines of the present investigation may be worth consideration as the rangemeter is at least a "valid and standardized technique" (Hammond, 1955). There is no reason why the rangemeter should not be developed as a carrier for an arsenal of other devices which would thus automize a deal of the work of grazing studies. For example, pressure time switches can be incorporated in the girth strap so that lying down periods can be measured and mapped within a day or week. The machine can also be modified to enable a ram to serve while wearing it. It has been improved by the writer and used successfully for breed and range studies in the United States (Cresswell & Harris, 1959).

Summary

The development of a new technique for sheep behaviour studies is discussed. The apparatus consists of a light harness, two shafts and a small trailing wheel which automatically records the distance travelled by an animal. This equipment was used in a comparative study of Romney Marsh and Cheviot sheep on hill and flat land in New Zealand. The results of this work indicate large breed differences in activity and reaction to varying environments. The records also contradict several of the ideas contained in modern theory on grazing behaviour.

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THE ELECTROENCEPHALOGRAM OF GOATS DURING SOMNOLENCE AND RUMINATION

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The physiological state recognized as "sleep" has been investigated in a number of different animal species and it is accepted that man and many other animals sleep profoundly with loss of consciousness (Kleitman, 1939). On the other hand it is well known that ruminants such as cattle, sheep and goats do not enter the state of deep sleep shown by other domesticated animals such as the horse, dog and cat.

Brownlee (1950), Hancock (1950) and Tribe (1950) made very close observations on cattle and sheep continually for 24-hour periods without noticing any loss of consciousness comparable to that shown by other species when they are asleep. Balch (1955), while making observations on gastric activity in cattle, also noticed that sleep did not occur although on occasions reduction in respiratory rate and slowing of the rate of contraction of the reticulorumen were apparent. He concluded that sternal recumbency is an attitudinal prerequisite for the proper functioning of the reticulorumen but that this posture prevents loss of consciousness associated with the induction of sleep. (Munro, 1957) observed that four, tame, bottle-fed lambs of approximately 18 months of age slept for short periods of up to 38 minutes' duration. She showed photographically that sheep can sleep apparently when lying down but with the head erect and the neck extended. The remarkable fact that ruminant herbivores do not sleep or at the best sleep very little, is in marked contrast to the horse for this species may sleep profoundly for seven hours out of the twenty-four (Steinhart, 1937).

Ruminants show periods of inactivity scattered sporadically over the twenty-four hours but occupying about eight hours in total time. During bouts of rumination cattle, sheep and goats are usually recumbent and show obvious signs of torpidity and although rumination can occur at any time of the day the greatest part of rumination time occurs at night (Bell & Lawn, 1957).

It has been possible to correlate the electrical activity of the brain in a number of species with various stages of sleep by means of the electroencephalograph (Simon & Emmons, 1956). This report describes the results obtained when electroencephalographic records are made from normal unrestrained goats during activity, rest and rumination.

Methods

Thirteen fully grown, castrated male goats have been used in the experiments. Each animal was fitted with a set of three or four unipolar electrodes while anaesthetized and with full aseptic technique. The placing of electrodes so that they impinged on to the surface of the cortex and electrode construction was similar to the methods described for the cat by Bradley & Elkes (1953). The parietal bone immediately behind the horn buds was exposed and drilled to accommodate the stainless steel screws carrying the electrodes; the insulated lead from each electrode was brought to the surface halfway along the dorsal surface of the neck. Usually four electrodes were implanted, the leads being soldered to a small plug which engaged in a socket connected to the amplifiers. For recording the animal was allowed free movement in a 4-ft. square cage or occasionally the goats were restrained in a Pavlov stand. The EEG was displayed by means of an Ediswan pen oscillograph.

Results

Electroencephalographic records from alert but recumbent goats show a low voltage, high frequency trace similar to the asynchronous records noted in other species (Fig. 1). Similar records can be made from goats restrained in a Pavlov stand. The asynchronous trace of the attentive animal contrasts markedly with the EEG recorded from the goat during thiopentone narcosis (Fig. 2), where the recording shows slow synchronized waves of high amplitude.

Goats often lie down but when recumbent they remain very alert, moving the head and ears continuously to scan auditory and visual stimuli. When the animals become drowsy they are usually in this recumbent posture but they do
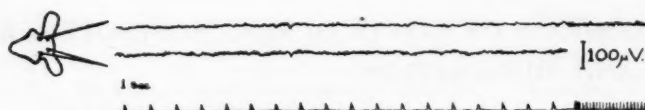
Fig. 1. Electroencephalographic records of the goat brain taken when the animal is in an alert state. In this and the following figures the position of the recording electrodes is shown on the diagram to the left of the tracings.
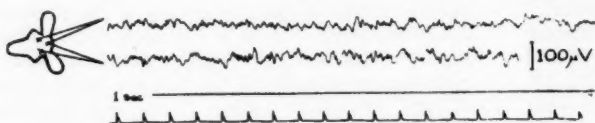

Fig. 2. EEG of goat after 20 mg/kg. thiopentone sodium. Palpebral reflex absent but corneal reflex brisk.

show additional attitudinal changes wherein the head is slightly lowered and the ears droop sideways away from the normal erect position. The EEG of the goat during this somnolent state is very similar to the pattern recorded from other animals during sleep and from goats during anaesthesia (cf. Figs. 2 and 3). The EEG shows low frequency high amplitude waves with spindle bursts which can be changed immediately to the asynchronous form when the animal is
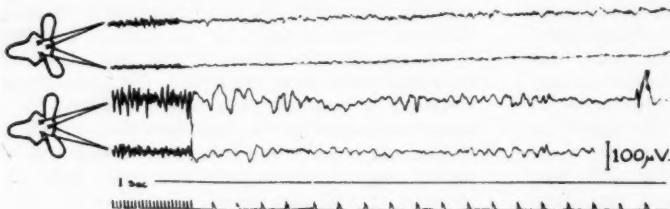

Fig. 3. Upper records show alert EEG taken 4 minutes prior to lower traces of goat in somnolent state. The animal is not asleep but it is lying down with the head held erect and with neck extended.
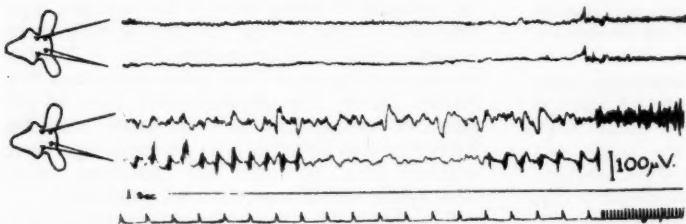

Fig. 4. EEG of goat during rumination. The middle part of the record shows the pause in remastication to allow swallowing and regurgitation. The pause is well illustrated in the lower trace by the interruption of the artefact produced by the muscle potentials associated with the regular chewing movements. The two upper records were taken 5 minutes before the lower traces and show the normal alert EEG.

alerted by auditory, visual or tactile stimuli. The more profound the state of somnolence, as measured by the attitudinal behaviour of the animal and by the regularity of the EEG, the greater the stimulus necessary to produce an arousal reaction. Auditory stimuli appear to be most important in affecting the arousal reaction in goats.

Immediately prior to and during rumination, animals show overt signs of drowsiness. They usually become recumbent and exhibit a general slackening of muscular tone. It is relatively easy to forecast the onset of rumination because of this change of demeanour, which is noticeable especially by an alteration in the animal's facial expression and in the carriage of its head. During rumination the EEG shows a pattern exactly similar to that seen during somnolence (Fig. 4). If the goat is alerted during rumination the hypersynchronous EEG is transformed to the normal active pattern of wakefulness. The degree of EEG change varies directly with the intensity of the alerting influence, an intense stimulus produces an "alert" type of EEG and inhibits rumination altogether. When an animal is ruminating in a profound somnolent state the EEG settles down to a low frequency, high amplitude pattern and the rumination cycles become prolonged and remain very regular at about 50-60 seconds' duration for long periods of an hour or more (see Bell & Lawn, 1957). During this settled period of rumination activity the animals are resistant to stimuli which would normally produce arousal possibly because the thresholds of the sensory receptors have been raised to a higher level. If the animal is constantly being alerted by sensory stimuli then the rumination cycles become shortened and irregular, they may then become intermittent or be abolished altogether (Fig. 5).
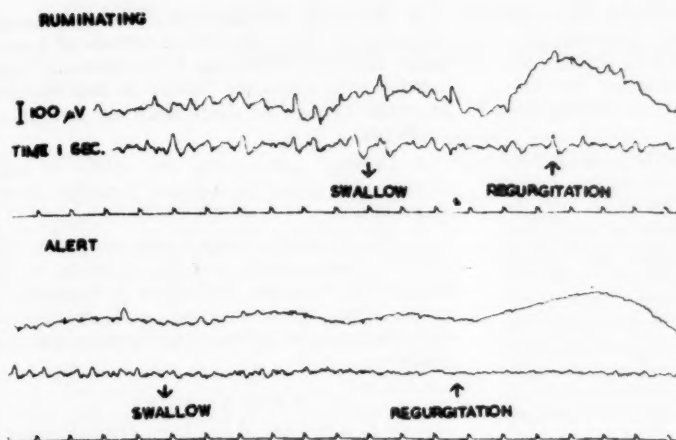
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Fig. 5. EEG of goat during rumination. In the upper traces rumination is being carried out with the animal in a profound somnolent state, with regular rumination cycles. During the recording of the lower records the animal was alerted by auditory stimuli; the rumination cycles became irregular and the periods between swallowing and regurgitation were prolonged. When the animal became quite alert rumination ceased.

the EEG showed synchrony comparable to the very deep sleep of other animals (see Simon & Emmons, 1956), the goats did not close their eyes or become laterally recumbent. The complete muscular relaxation which is a feature of somnolence in most species does not occur in the goat.

Discussion

The results obtained from these experiments corroborates the view that members of the Bovidae do not sleep in the same way as do other animals. This behavioural characteristic may be associated with the anatomical and biochemical differences shown between the Bovidae and other species. Balch (1955) has suggested that because of the anatomical disposition of the ruminant stomach and the continuous activity of the reticulum, these animals are precluded from adopting the normal posture of sleep which is one of lateral recumbency.

The findings reported here that ruminants (goats) do show cerebral electrical activity which is normally associated with the state of deep sleep is of great interest because the animals do not lose consciousness. During somnolence, however, when the EEG is of an hypersynchronous type the animals show some signs which are often associated with sleep. For example, the animals usually lie down and the re-

cumbency is followed by some loss of muscle tone so that the carriage of the head is lowered and the ears droop away sideways. The thresholds of sensory stimuli are also raised during these periods of languor of somnolence and rumination. The somnolent ruminant would appear to be cut off from its environment because of the rise in receptor threshold, but to a much less degree than other animals when a state of unconsciousness develops with sleep. The ruminant, moreover, during somnolence has its eyes open which is not normally an accompaniment of sleep.

The state of wakefulness and the state of sleep are now regarded as being associated with the level of nervous activity of the diencephalon (Hess, 1949; Magoun, 1950). It is now agreed that the balance between sleep and wakefulness is co-ordinated at the diencephalic level, Hess postulating a "sleep" centre which can be activated by an ergotropic centre of the posterior hypothalamus. Magoun (1954) ascribes sleep and wakefulness to the varying activity of the ascending reticular formation, activation of this sensory system producing arousal. In the goat, judging from the synchronous nature of the somnolent EEG and the asynchronous nature of the alert EEG, it would appear that a similar neural control mechanism applies.

The association of rumination with the state of somnolence and an EEG similar to that of sleep in other animals is of interest because goats do not exhibit overt signs of sleep at this time. In goats the great amount of rumination activity occurs at night when darkness would deprive the animal of a large amount of stimulation from all sources but especially those of visual and auditory origin (Bell & Lawn, 1957). This diminution of sensory traffic would produce minimal activity in the ascending reticular formation so that the brain potentials would become synchronized. The neural activity which allows mastication, swallowing and regurgitation as well as the rapid muscular contraction of the reticulum during the somnolent state with a

sleep-like EEG would thus appear to be a contradiction. It has been noticed, however, that one of the signs of sleep in man is increased tone and activity of the orbicular muscles and that some automatic processes may be carried out during somnolence.

Not all animals become recumbent when they begin to ruminate nor does rumination occur exclusively at night. For example cows congregated at the field gate prior to milking very often ruminate. It is possible that some mechanism other than diminution of sensory impulses allows the mechanisms which co-ordinate rumination to function. For example, narcotics and anaesthetics may suppress the ascending reticular formation and produce loss of wakefulness (French, Verzeano & Magoun, 1953). Very often cows recovering from narcosis begin to ruminate in rather a desultory fashion. Diminished activity of the reticular system also occur with variation of metabolic activity such as hypoglycaemia and oxygen depletion (Arduini & Arduini, 1954). During digestion in the ruminant large quantities of vegetable material yield large volumes of gases such as hydrogen, methane and carbon dioxide as well as a variety of short-chain fatty acids. It is possible that one, or a combination of more than one, of these products of metabolism gains access to the blood in sufficient amount to cause reversible blockage of the ascending reticular formation.

The stimulus which induces an animal to begin ruminating or to stop ruminating has not, as yet, been ascertained. It appears likely from the foregoing experiments that rumination is closely associated with somnolence and that the state of somnolence in ruminants is controlled through the activity of the ascending reticular formation of the brain-stem. In order that rumination can ensue it is probable that the ascending reticular formation must be blocked or its activity drastically suppressed. Even when the ascending reticular formation is not active with a resultant synchronization of the EEG, ruminants do not show the prolonged periods of unconsciousness which is a characteristic of sleep in most animals.

Summary

1. The EEG of alert goats is similar to the asynchronous pattern noted in other species.

2. Goats do not sleep like other non-ruminant species. They do exhibit periods of somnolence, however, when the EEG shows a hypersynchronous chaiacter similar to that recorded in other species in deep sleep or during anaesthesia.

3. During rumination the EEG is indistinguishable from the pattern recorded during somnolence.

4. The possible connection between somnolence, rumination and the activity of the brain-stem reticular formation is discussed in relationship to anatomical and biochemical differences shown between the Bovidae and other species.

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A STUDY OF SEX DRIVE OF TWO STRAINS OF COCKERELS THROUGH THREE GENERATIONS

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Guhl (1951) examined the mating frequencies of a number of cocks and found that they differed significantly. Wood-Gush & Osborne (1956) in an attempt to discover the causal mechanisms of such differences examined the mating frequencies of thirty males belonging to six different sire families. Significant differences were found in the rates at which the males mated. Furthermore, the differences between the families with respect to this trait were also found to be significant, indicating that the trait has a genetic basis, although in this analysis the dams failed to make a significant contribution to the genetic variance. Semen samples collected monthly from each male during the three months of the experiment were assessed and graded on the basis of volume, sperm density and sperm morphology. From this there appeared to be little or no relationship between a cock's ranking for sexual activity and the grading of his sample. In order to ascertain whether there was any positive correlation between the frequency of mating and ability to inseminate hens, five males with highest scores for mating frequency and the five lowest scoring males were tested, and it was found that four of the low scoring males failed to inseminate any females while the top scoring males all inseminated females. A significant negative correlation was found between comb height and mating activity; the males which had the largest combs tended to have the lowest scores for mating activity. In order to test whether the possession of a large comb was lowering the sexual libido of birds by generally impeding their movements, two large combed birds were dubbed. After a five week convalescence during which food was supplied *ad lib.* they were again tested. Although a little improvement in their performance was noted, they still differed significantly from the two high scoring birds used as controls. These findings suggested that the large combs were unlikely to be the primary cause of their low mating scores.

Experiments with F_1 Generation

An F_1 generation was bred to verify that the differences in mating frequency in this population were under genetic control and to ascertain whether the female parent could make any significant contribution to the genetic variance of this trait. The four top scoring males and the four lowest scoring males were used as sires. The breeding programme is shown below in Table I. Each female was inseminated first with the semen of either a high or low scoring male, and then after a suitable lapse, with the semen from a male of the other type.

Table I. The Breeding Programme Employed to Obtain the F_1 Generation Males.

Dam	High scoring sire	No. of male offspring produced	Low scoring sire	No. of male offspring produced
1	A	2	W	1
2	B	1	X	1
3	B	3	X	2
4	C	2	Y	1
5	D	1	Z	3
6	D	1	Z	1
Total		10		9

The F_1 males were all dubbed as day-old birds to eliminate any comb effect. Their rearing and maintenance followed the pattern of the P_1 males (Wood-Gush & Osborne, 1956) and the method of testing them was essentially the same except for the fact that they were three months older. Before being tested for mating frequency each male in the two generations was given two 48-hour periods alone in a pen with a number of hens in reproductive condition. This constituted their first heterosexual experience. Then, after a 48-hour rest, each was placed individually in an observation pen with a standard flock of females, and all matings during one hour were recorded. At this point a difference between the two generations emerged. Whereas none of the P_1 males had been aggressive towards the females in the observation pen, a number of F_1 males

behaved aggressively towards the females. After a period of aggressiveness towards the females these males were then usually indifferent towards them, and only after this phase did sexual activity commence.

Any F_1 males that had not mated after their two 48-hour exposures to females were then given further 48-hour exposures and after a suitable rest were tested again after each exposure. It was found that the sons of males with high libido scores ("High Strain"—H.S.) took, on the average, 4·6 days to their first mating while the sons of males with low libido scores ("Low Strain"—L.S.) took 11·7 days. An analysis of variance performed on the data showed that these differences between the two strains were significant at the 1 per cent. level.

After each male had mated he was then given a total period of 14 days alone with a group of hens to become fully adapted to them, and when all the males had undergone this treatment they were tested for mating activity in each of two observation pens. The results from these tests are shown below in Table II.

The data were then analysed according to the analysis variance also shown in Table II.

These data confirm the earlier finding of Wood-Gush & Osborne (1956) that the differences in mating frequencies of the males in this population are under genetic control, and the analysis also reveals that the dam had made a significant contribution to the genetic variance of the trait. Furthermore, the persistance of the differences between the two strains of cockerels which had been dubbed as day-old birds supported the earlier finding that the performance of the low-scoring males was not determined only by possession of large combs.

The fact that the L.S. males had taken significantly longer to adapt themselves to females suggested that they might be generally more aggressive towards other birds than the H.S.

Table II. The Total Number of Matings Performed in 4 Tests in 2 Pens, and Analysis of Variance of Mating Frequencies of the F_1 Males from the Two Strains.

Dam no.	"High Strain"			"Low strain"		
	Cockerel no.	Sire	Total no. of matings	Cockerel no.	Sire	Total no. of matings
1	112	A	8	7301	W	7
	220	A	15			
2	7169	B	19	6602	X	10
3	6802	B	11	6699	X	3
	6901	B	12	6696	X	1
	7160	B	18			
4	7165	C	12	3	Y	7
	7302	C	15			
5	6598	D	8	6904	Z	1
				7029	Z	4
				7162	Z	4
6	417	D	11	7034	Z	18

Source of variance	Degrees of freedom	Mean Square
Between dams	5	44·3*
Between strains	1	218·31**
Interaction between dams and strains	5	14·09
Error	7	9·38

*   indicates P 0·05
** indicates P 0·01

cocks, and that their sexual libido potential was being masked by their aggressiveness. All the males from the two strains were therefore tested and scored for aggressiveness by a system described fully elsewhere (Wood-Gush, 1957). No correlation was found to exist between the scores for aggressiveness and the scores for mating frequencies; the two strains did not differ in their aggressiveness towards other males. Nevertheless the possibility remained that the differences in mating frequencies were due to differences in the abilities of the males to adapt themselves to females, for while relatively late sexual experience had overcome aggressiveness towards females, the L.S. males might not have been able to express fully their sexual potentialities. The period of isolation from females, from puberty to testing, might have had a detrimental effect on their sex drive. Although none of the P_1 males had shown overt aggressiveness towards the females, some had been indifferent towards them; two full brothers, for example, had not courted the females or attempted to mount them. Thus it seemed feasible that some of the males of this P_1 generation had still been in the indifferent stage when tested for mating frequency. The absence of aggression in the P_1 males towards females under this hypothesis might have been due to their being tested at a younger age than the F_1 males, i.e. their period of isolation from females had been shorter.

Experiments with F_2 Males

In order to test the above mentioned hypothesis (i.e. that the differences in mating frequencies really reflected genetic differences in the ability of the cockerels to adapt themselves to females when six months of age or older) an F_2 generation was raised and half the males of this generation was allowed controlled access to females from an early age.

1. Breeding and Rearing

A breeding programme very similar to that carried out to obtain the F_1 generation was used for breeding the F_2 males. Unfortunately due to lack of space no F_1 females had been reared so an unrelated stock of females was used for breeding. The two top scoring high strain males and the two lowest scoring low strain males were used as sires and each female was inseminated with the semen of either a H.S. sire or L.S. sire and then with semen from a sire of the other strain when she ceased to give fertile eggs from the first insemination. Twenty-four F_2 males (12

from each "strain") were then used for the following experiments. They were reared in brooders until six weeks of age and then placed in two pens without females until six months old. At the age of 13-14 weeks, twelve of the males, six from each strain, were placed individually in a pen of pullets aged twelve weeks, for a period of one day every two weeks. During the first twenty minutes of their release into the pullet pen, each male was observed and full notes on his behaviour were taken. After removal from the pullet pen, the male was then returned to his pen-mates which included males that were not undergoing this experience. In this way it was hoped to eliminate any difference in experience between the males that were receiving early sexual experience and those that were being reared in the "normal" way.

2. Observations on Mating Behaviour

Shortly after the commencement of this experiment one H.S. male became ill and so was excluded from the experiment, leaving five H.S. males and six L.S. males in the group with access to females from the age of 13-14 weeks. As stated, these males were under observation for the first twenty minutes after their release into the pullet pen, and the number of attempted and completed matings during their first four exposures to pullets are shown (Tables III and IV). Semen samples were taken from each male after their second and fourth exposures when they were approximately 17 and 21 weeks old. No differences in courtship between the males of the two strains were apparent and all were extremely tolerant to the young pullets which pecked at their feathers, rings and plastic wing bands.

The data from these two tables (III and IV) indicate that generally the H.S. males were more precocious in their sexual behaviour, although both lots of males were very tolerant towards the pullets. The L.S. males, however, appeared to be more precocious in semen production, a point to be discussed later.

After these males had had their fourth exposure to the pullets all the twenty-three males in this experiment were given two 48-hour exposures to females as the P_1 and F_1 males had received (no other males were present during the exposures). After the second 48-hour exposure the males that had not received any previous sexual experience were then released individually into observation pens containing a standard number of females in reproductive condition. None of the males was aggressive towards the

Table III. The Sexual Behaviour and Semen Production of "High" and "Low" Strain Males Exposed to Pullets of Slightly Younger Age. The number of matings shown in the table constitute the total number of matings observed during their first two exposures to the pullets, and the semen samples were taken when the males were approximately 17 weeks old.

Strain	Cockerel	Sexual behaviour	Semen grading	Body weight
	11	No mounting	Copulatory organ erected Little semen	1460 gm.
	24	5 mountings	No erection. No semen	1620 gm.
"High"	25	6 mountings	Weak erection. No semen	1315 gm.
	26	3 mountings	No erection. No semen	1340 gm.
	12	1 mounting	Weak erection. No semen	1700 gm.
	16	2 mountings	Copulatory organ erected. Little semen	1740 gm.
	17	1 complete copulation	Erection. Little semen	1625 gm.
"Low"	14	Nil	Erection. Semen watery	1340 gm.
	15	Nil	Erection. Semen watery	1360 gm.
	22	1 mounting	Erection. Yielded semen	1315 gm.
	30	1 mounting 1 complete copulation	Weak erection. Very little semen	1550 gm.

Table IV. The Sexual Behaviour of "High" and "Low" Strain Males During Their 3rd and 4th Exposure to Pullets of Slightly Younger Age. The semen samples were taken when the males were approximately 21 weeks of age.

Strain	Cockerel	No. of matings (complete and incomplete)	Semen grading	Body weight (grammes)
	11	Nil	Adult type	1760
	24	7	,, ,,	1810
"High"	25	6	,, ,,	1590
	26	12	,, ,,	1590
	12	12	Weak erection. Little milky semen	1800
	16	2	Adult type	1980
	17	Nil	,, ,,	1720
	14	1	,, ,,	1690
"Low"	15	1	,, ,,	1600
	22	4	,, ,,	1490
	30	4	,, ,,	1800

females and all copulated. This finding suggested that the aggressiveness of the F_1 males towards the females was due to the fact that they had been isolated from females for a longer time than the P_1 and F_2 males.

Before proceeding to test all the males for frequency of mating, the eleven males that had had previous experience were tested individually in each of two observation pens. Cockerel No. 11 of the H.S., which had not been at all precocious,

now proved to be the highest scoring male, and the H.S. males proved to be superior in this test to the L.S. males. The final tests then began: all the males were tested once in each of the two observation pens. The females were all in reproductive condition and sexually experienced. Each male was tested individually and the observer was hidden at all times (for fuller details of the technique see Wood-Gush & Osborne, 1956). The mating scores are shown below (Table V).

female pen. In other words the males from both strains were equally quickly motivated to mate.

3. Other Behavioural Differences

When the observations on sexual behaviour had been completed, the males were then subjected to a number of simple tests to determine whether the two strains differed in other behavioural traits, or whether the difference in sexual behaviour was the only major difference

Table V. The Total Number of Matings (Complete and Incomplete) Performed by All Males in Each of Two Observation Pens.

High Strain				Low Strain			
Males with early sexual experience		Control males		Males with early sexual experience		Control males	
No. of males	Total no. of matings	No. of males	Total no. of matings	No. of males	Total no. of matings	No. of males	Total no. of matings
11	18	1	13	16	11	6	8
24	11	2	10	17	4	8	9
25	9	3	10	14	5	4	2
26	11	9	15	15	5	5	4
12	11	10	11	22	6	21	8
		27	10	30	8	28	5
Total	60	—	69	—	39	—	36
Mean	12		11·5		6·5		6·0
High strain mean and std. error 11·7 ± 0·7979				Low strain mean and std. error 6·25 ± 0·7399			

The data given in Table V indicate that early sexual experience has been without effect on the mating frequencies of the males of the two strains. Furthermore the differences in the mating frequencies of the males from the two strains are significantly different. Since all the males were very tolerant towards the females and as half the L.S. males had received optimum conditions under which to adapt themselves to females; it seems unlikely that the differences in mating frequencies between the two strains are due to differences in the rates at which males adapted themselves towards the females after a period of heterosexual isolation. The causation of these differences should be sought in some other mechanism. A further examination of the records showed that there was no difference between the two strains in the time taken to the first mating from the time of entry into the

between the two groups. The first of these tests, (a) Aggressiveness and (b) Crowing, are, in common with sexual behaviour, characteristic of males in reproductive condition, and affected by androgen. However, whereas aggression is aroused during sexual behaviour, a male seldom crows then, nor does a dominant male attack or prevent his inferiors in the same pen from crowing. It seems to that extent independent of specifically sexual or aggressive behaviour notwithstanding the function ascribed to it by Collias & Joos (1953): that of attracting females and warding off males.

The next two tests, (c) and (d), were designed to investigate possible differences between the two lines in respect of some behavioural characteristics which do not depend on the reproductive or sexual condition of the bird.

Together these tests could be expected to give

an indication of the degree of behavioural specificity of the difference between the lines, and perhaps provide some clue as to the causal mechanism underlying it.

a. Aggressiveness

The method used for testing this trait has been described fully elsewhere (Wood-Gush, 1957). Briefly it consists of testing each male against a member of a panel that has been trained to fight. Two measurements were taken at each encounter: 1. the latent time or the time taken to the start of a fight; 2, the number of fights initiated, allowing one fight per encounter. The tested bird was given two points for starting the fight, one if both started the fighting and 0 if the panel member started the fight. This is defined as the "fighting score". Once fighting had started the birds were immediately separated to prevent any conditioning effect. The mean latent time scores for the H.S. was $117 \cdot 09 + 45 \cdot 1$ seconds and $102 \cdot 66 + 78 \cdot 4$ seconds for the L.S. The mean fighting scores for the "H" and "L" strains were $3 \cdot 27 + 0 \cdot 41$ and $3 \cdot 97 + 0 \cdot 34$ respectively. In neither of these scores do the two strains differ significantly. This finding is in agreement with the data from the P_1 and F_1 generations.

b. Crowing

In this test the males were released singly into a strange empty pen from which other birds could only be seen from certain points. The number of crows made by each male from the two strains were counted over a twenty minute period. To eliminate effects from the crowing of other birds, the observations were done at the same time of day and notes were taken of any other birds that could be heard. The mean number of crows by the males of the H.S. and L.S. groups was $20 \cdot 6 + 2 \cdot 14$ and $18 \cdot 8 + 3 \cdot 098$ respectively. In other words the males of the two strains did not differ in this measurement of "maleness."

c. Acceptance of Unusual Food-stuffs

In this test the birds were offered, after some starvation, strange looking food-stuffs. Each bird was starved for twenty-four hours and then tested singly in an empty pen. On the bare cement floor of a pen, at a set position relative to the door, was placed a small pile of 20 normal wheat grains and on one side of this pile was a heap of 100 wheat grains that had been dyed red and at an equal distance on the other side was a pile of 100 blue wheat grains. The majority

of males consumed the normal grains first and then continued with either the red or the blue grains, if they were going to continue. However, two H.S. males and one L.S. male discriminated on quantity rather than colour during repeated tests, and were eliminated from the experiment in case they were colour blind. In the H.S. group there were, after this correction, nine apparently non-colour blind males and two of these totally rejected any coloured wheat grains. Of the eleven apparently non-colour blind males in the L.S. groups, seven totally rejected any coloured grains. Assuming that these rejecting males were equally distributed between the two strains, a chi-square test was applied. The actual numbers of rejecting males in both strains did not differ significantly from the expected numbers assuming equal distribution between the strains.

A closer examination of the data reveals an interesting point: of those birds, from both lines, which did take coloured grains, the H.S. males more frequently tended to try grains of both colours than did the L.S. males; and they tried a total of eleven coloured piles while the L.S. males attempted only five. These differences were nearly significant at the 5 per cent. level.

d. Alarm Calls in Strange Situation

Broadhurst (1957) used frequency of defaecation as an index of fear in mice. Since alarm calls appear to be a better index of fear than defaecation, an attempt was made to use the latter as such an index. A male in a strange situation will often give alarm calls of very high intensity without defaecation, while a male that gives no alarm calls will defaecate readily. A small pilot experiment was carried out on the time given to alarm calls in a strange situation during a three minute period, but the results showed that the three males from each strain were very different from each other and no conclusions were drawn. This is mentioned for those who are interested in this type of score for this species.

4. Physiological Tests

a. Measurement of Secondary Sexual Characters

It will be recalled that on the P_1 generation, a significant negative correlation had been found between comb size and mating frequency. The F_1 and F_2 males had all been dubbed as day-old birds and so no comb measurements were possible, but wattle measurements of the F_2 males revealed that the L.S. males had longer wattles although there were no differences in body weights between the two strains. The H.S.

mean body weight was $1.957 \pm .025$ kg. and the L.S. mean was $1.974 \pm .0357$ kg. The wattle length means were 66.2 ± 3.13 mm. and 79.5 ± 1.71 mm. for the H.S. and L.S. males respectively. This finding suggested that the main differences found in the initial population were still extant in the F_2 generation. Furthermore, it suggested that the differences between the sexual behaviour of the two strains might be connected with some hormonal state, either a difference in production or in the response of the C.N.S. to hormone levels.

The chick comb has been used for a long time to assay androgens and so the evidence seemed to warrant an investigation to determine whether the differences between the two strains were due to some variation in their androgenic output, or their response to androgens.

It is well known that cockerels of different genetic backgrounds (e.g. breeds) differ a great deal in the response of their combs to exogenous androgens. The possibility existed, *inter alia*, that the combs of the L.S. males were more sensitive to androgens than those of H.S. males, while that part of the C.N.S. mediating sexual behaviour was less sensitive in the L.S. males. The main question, then, was to try to find if there were any differences in the amount of androgenic substances in the males of the two strains. Enormous difficulties arise when this question is to be answered. No estimations of 17-keto-steroids from urine are possible in the chicken. Techniques using the blood seem to be very unpromising. An unsuccessful attempt was made to recover testosterone from the testes using the technique of Koenig, Melzer, Szega & Samuels (1941). Furthermore, it is not known whether the chicken does in fact have testosterone or some homologue. It is possible that it is there in some bound form difficult to recover. Therefore, a number of physiological tests were carried out which it was known were influenced in a very crude way by the presence of androgens or oestrogens and which would, taken together, give an indication of any large differences in androgen levels if these were present in the two strains.

b. Testis Weight

Although the weight of an organ cannot give an accurate measurement of its activity, large differences in testis weights might help to reveal further differences which could be studied. No differences were found. The H.S. mean was 16.74 ± 1.45 gms., the L.S. mean was 16.19 ± 0.97.

c. Creatine Concentration of Seminal Plasma

Greenwald (1946) investigated the creatine content of the testis of various marine invertebrates and found that the concentration increased as testicular activity waxed. It was considered therefore that the creatine concentration of the cock's seminal plasma might reflect the metabolic activity of the testis although some of it might be derived from the spermatozoa. Techniques have been described by Lake & McIndoe (1959). No differences were found. The means for the H.S. and L.S. groups were 104.9 ± 5.61 mg./100 ml. seminal plasma and 96.6 ± 5.6 mg./100 ml. respectively.

d. Packed Cell Volume

When the volume of the blood cells is read in chickens after centrifuging the blood, it is found that there is a general difference between the two sexes in this measurement which becomes evident at about the time of puberty. In young chicks, testosterone propionate injections influence the packed cell volume, whereas stilboestrol is ineffective (Newell & Shaffer, 1950). No differences between the strains were found.

e. Liver Clearance

The clearance of the dye sodium bromosulphthalein by the liver of the chicken, shows that there is a difference in the clearance gradient of the two sexes. Stilboestrol caponized males show a female type clearance (Campbell, 1957), and the test might be considered to be indicative of the oestrogen/androgen balance of a bird. No differences between males of strains were found although there was a great deal of variability within strains.

f. Response to Exogenous Testosterone Propionate

Several workers, e.g. Craig, Casida & Chapman (1954) using mammals, have improved the libido of males by the use of exogenous testosterone. Therefore, it was decided to try an experiment using testosterone propionate to see if the sexual vigour of the L.S. animals could be improved. In supplying a exogenous testicular hormone, it is to be expected that the endogenous supply will be decreased but that eventually the amount of hormone introduced will exceed the quantity normally present, and the subjects will behave as though the gland is overactive. This has been demonstrated with thyroxine. Large doses caused atrophy of the gland together with symptoms of hyperthyroidism in

the subjects (Dempsey, 1951). From a pilot experiment performed with other cocks of similar age it was found that about 10 mg. testosterone increased the mating frequency of the treated males. In this experiment, if the males of one strain should increase their mating scores significantly after a certain dosage it might be assumed that the dosage rate had surpassed the rate of production of the endogenous hormone, and in this way a crude measurement of its production might be derived. Conversely, however, the lack of a significant increase does not mean that the native hormone production has not been exceeded.

In the test carried out eight males from each strain were used. Four from each strain received 10 mg. of testosterone propionate in three doses of 4, 4 and 2 mg. Each male was tested in the late afternoon of the day on which the last dose was given; the doses having been injected between 10-10.30 a.m. each day. The other eight males served as controls. A higher score was achieved by the treated males in both strains, but the increase in the treated L.S. males did not approach the score of the H.S. males, whether treated or untreated. Furthermore, two of the treated L.S. males received a further 10 mg. and were again tested but their scores after 20 mg. of testosterone propionate were identical with their scores achieved after 10 mg. The results of the test are shown in Table VI.

Table VI. The Effect of Testosterone Injection on the Mating Frequency of High and Low Strain Males.

High strain	Treated males	22 matings
	Control males	18 matings
Low strain	Treated males	7 matings
	Control males	3 matings

Although the increase in mating frequency was relatively greater in the L.S. males compared with the H.S. males, the results do not suggest that the difference in mating frequency between the two groups was entirely due to differences in the production of testosterone or its homologue.

In the absence of direct methods for qualitative and quantitative assays on the hormones of these birds it is impossible to say whether subtle endocrine differences exist between the two strains, but the present data would rule out the possibility of gross differences in the oestrogen/androgen balance between the two strains.

g. Semen Production

In the P_1 generation there had been no correlation between mating frequency and volume of semen yield by means of manual massage, but in the F_1 generation there was a slightly greater number of poor donors among the H.S. males than among L.S. males. In the F_2 generation, however, L.S. males proved to be remarkably better donors. Ten out of these twelve males were scored as good donors by a very experienced collector, and the remaining two were classified as average donors. Of the H.S. males only two were graded as good, four as average, and the remaining five were classified as poor donors. These results suggested that the causal mechanisms of the differences in mating frequency might be connected with the semen production of ejaculatory patterns of the two strains. In order to study this possibility another generation of males (F_3) was bred. The two top scoring males from the H.S. group and the two lowest scoring males from the L.S. group were used as sires, while the dams of this F_3 generation were three females related to the H.S. F_2 males and three related to the L.S. F_2 males. No cross matings were made. Twelve male progeny from each strain were chosen at random to be test animals for this generation.

These F_3 birds were reared in communal brooders until eight weeks of age and then removed to pens until six months old, when they were caged individually. The males from the two strains were mixed in the pens. At six months of age these males were tested three times for semen production, using manual massage. Again the L.S. males were superior in this respect. The mean volumes for the H.S. and L.S. groups were 0.16 ± 0.046 ml. and 0.455 ± 0.043 ml. respectively.

Shortly after the tests were started, a nephritic ailment occurred in the two strains, and three males of each strain died. Furthermore, when the remaining males were tested for mating activity, it was found that a number were very inconsistent and that the H.S. males were generally inferior, in mating frequency, to those in the previous generation: their score was now barely superior to that of the L.S. F_3 whose behaviour, however, was very similar to that of those of the previous generation.

After the remaining males had recovered they were again tested for semen production, and seven of the H.S. and six of the L.S. males were then found to be producing semen at approximately the same rates as before their illness. Their

mating behaviour too was rather more consistent than that of others, and so these males were used for the following observations, which, due to the small samples, can only be claimed to be indicative.

Some of the specific questions raised from the F_2 data which appeared to be open to experimentation and which would then allow further theoretical considerations to be raised were:

1. Is there any difference in the rate at which the males from the two strains reach exhaustion in semen production? The higher mating frequency of the one strain might depend upon their ability to produce semen at a quicker rate albeit in smaller quantities.

2. Is there any difference in the amount ejaculated at each apparently complete copulation by the males of these strains?

Exhaustion Tests

Five males from each strain were "milked" for semen by manual massage four times within 65 minutes. The results are shown in Table VII.

iately after his first complete mating, i.e. one in which there was cloacal contact, the female was caught, her oviduct everted and a smear was taken. If no semen was apparent, then a small pipette was inserted into the oviduct in an attempt to recover any semen that might be there. Five H.S. males and four L.S. males were tested in this way. Three of the H.S. males were tested three times and two were tested twice. The L.S. males were each tested three times. All repetitions were done on different days. The results are shown in Table VIII.

It is apparent that the first copulation of the

Table VIII. The Numbers of First Copulations with, and without, Ejaculation by Males of the Two Selected Strains.

	No. of first copulations without ejaculations	No. of first copulations with ejaculations
High strain	7	6
Low strain	1	11

Table VII. Volume of Semen in ml. Produced by Individual Cockerels (Indicated by their Number above the Columns) at 4 Successive Twenty-minute Intervals from 16.25 to 17.29 hours.

Low strain males					High strain males					Time
6	20	34	23	39	32	13	44	24	3	
0·32	0·34	0·95	0·21	0·31	0·23	0·10	0.02	0·33	0·28	16·25—16·40
0·27	0·37	0·16	0·05	0·13	0·02	0·02	0·02	0·11	0·10	16·45—16·56
0·12	0·15	0·13	0·02	0·13	0·02	0·04	0·02	0·10	0·04	17.05—17.15
0·07	0·15	0·11	Nil	0·04	0·05	0·03	0·02	0·06	0·04	17.20—17.29
0·78	1·06	1·35	0·28	0·61	0·32	0·19	0·08	0·60	0·46	Total

These data show that H.S. males do not generally produce as great a volume of semen as L.S. males over a limited period, but neither do they appear to approach exhaustion more rapidly during this period: they also suggest that the inferiority in mating frequency of the L.S. males is not connected with an inability to produce semen for another ejaculation shortly after the first.

Recovery of Male Semen from Hens

All these tests, like the exhaustion tests, were done in the late afternoon, when mating is most frequent. Each male was released singly into a pen of individually marked females and immediately after his first complete mating, i.e. one in which there was cloacal contact, the female was caught, her oviduct everted and a smear was taken. If no semen was apparent, then a small pipette was inserted into the oviduct in an attempt to recover any semen that might be there. Five H.S. males and four L.S. males were tested in this way. Three of the H.S. males were tested three times and two were tested twice. The L.S. males were each tested three times. All repetitions were done on different days. The results are shown in Table VIII.

H.S. males is frequently without ejaculation while the converse holds for males of the other strain. Furthermore, when the second copulations of the five H.S. males were examined in this way, semen was found in every case.

Discussion

It seems that in this particular population the H.S. males employ more cloacal contacts before ejaculating, and as a result their mating frequency scores will be higher than that of the other males for an equal number of inseminations. It was previously stated that these F_3 males, probably as a result of nephritis, were inferior to the H.S. males of the previous gener-

ations in mating frequency, and so the question immediately arises as to whether the frequency of copulations without ejaculation was a result of this illness. Parker, McKenzie & Kempster (1940) observed the mating behaviour of two groups of cocks, which had been under different environments and which differed in their mating frequencies. Each cock had a semen collector attached to the cloaca, and these workers found that in the group with the higher mating frequency the incidence of complete copulations without ejaculation was higher than in the other group, although the more sexually active group yielded large semen samples. Unfortunately no figures are cited on this point, but this evidence indicates that copulation without ejaculation does occur in normal healthy cocks, that the figures quoted above were not determined by the nephritis, and that much the same sort of behaviour was likely to have occurred in the previous H.S. generations, especially as the data of Parker *et al* (1940) show that copulation without ejaculation is not limited to birds with low semen production. This finding helps to explain the differences in mating frequency between these two groups in successive generations, but it may not be an entire explanation for in the first three generations of this investigation, the incidence of incomplete matings was always higher in the H.S. males. Furthermore, Parker *et al* (*loc. cit.*) also found that the incidence of incomplete matings was greater in their more sexually active group of cocks.

The greater number of intromissions before ejaculation in the H.S. cocks may be explained in a number of ways. They may be more easily motivated by the presence of females to initiate copulation, although their systems may not be entirely prepared for ejaculation. They may be less able to ejaculate than the others and therefore they may have to mount females more frequently in order to ejaculate. Under this latter hypothesis, the inhibiting factors might be peripheral and be dependent on the sensitivity of peripheral organs or they might be referred to the central nervous system. The present data are insufficient to test adequately these or other hypothesis.

Finally, a point of general interest raised by these experiments concerns the measurement of sex drive. If mating frequency is taken as the sole criterion, then one strain is definitely superior to the other. If semen production is considered as the criterion, this strain is then inferior.

Summary

A population of thirty cockerels belonging to six sire families was tested for mating frequency and differences between the families were found to exist.

The highest and lowest scoring males were chosen to sire the F_1 generation. The sons of the high scoring males (H.S. males) scored higher frequencies for mating activity than the sons of low scoring males (L.S. males). Furthermore, the H.S. males adapted themselves to females in a significantly shorter time than the L.S. males.

An F_2 generation of H.S. and L.S. males was bred following the same programme as that for obtaining the F_1 males. Half the males from each strain were allowed access to young females from an early age, but this treatment did not affect the differences in mating frequency between the two strains; the H.S. males were superior to the L.S. males regardless of early experience.

In the P_1 and F_2 generations an inverse relationship between the size of the head appendages and mating frequency was observed. No measurements were taken in the F_1 generation. The F_2 males were subjected to a number of physiological tests which were capable of indirectly discerning any large differences in the endocrinal output of the birds. No such differences were apparent. The birds were also examined in a number of other behavioural traits, but mating frequency appears to be the major behavioural difference between the two groups.

The L.S. males in the F_2 generation were better semen donors than the H.S. males, and this was also found in an F_3 generation. In this last generation it was found that the H.S. males had more intromissions per ejaculate than the L.S. males. The significance of this finding is discussed.

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PREFERENCE TESTS AND THE SENSE OF TASTE IN THE FERAL PIGEON (*Columba livia* Var Gmelin)

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Introduction

It is generally believed that the sense of taste in birds is poorly developed (see Sturkie, 1954) and only small numbers of taste buds have been reported in pigeons (Moore & Elliot, 1946) and bullfinches (Duncan, 1960). The maximum number of buds in both these species is about 50 to 60 in adult birds. Engelmann (1940 and 1943) concluded that hens base their preferences for certain cereals primarily on shape and secondarily on colour, whilst he found that taste had little effect. However, Kare, Black & Allison (1957) have described preference tests in which groups of 20 chicks were given a free choice of fluid from one of two drinking bowls. One of these "waterers" contained the flavoured solution and the other contained water. They report evidence of wide taste preferences for a variety of flavours.

In the present series of experiments feral pigeons (*Columba livia* var Gmelin) were used and other publications (Duncan, Wright & Ridpath, 1960; Duncan, 1960) have described the previous investigations on the sense of taste in this species. This paper gives a preliminary report of the preference tests which were carried out using feral pigeons. The birds were all mature and had therefore previously experienced a variety of tastes, a factor which would influence their preference or rejection of various substances.

Methods

Preference tests are generally designed to allow the animal a free choice between two drinking fountains, one containing water and the other the solution under investigation. Unfortunately the animals frequently prefer to drink from the fountain which is in a certain position in the cage and precautions have to be applied to eliminate this bias to the results, (see Kare & others, 1957).

In the present series of experiments the method of single stimuli developed by Beebe-Center, Black, Hoffman & Wade (1948) and Weiner & Stellar (1951) was adapted for use with pigeons. In this way there are no problems associated with the position of the drinking fountains. The birds were housed in separate cages and were unable to see one another. They were subjected to a regular, artificial day and night, and the room temperature was thermostatically regulated as far as possible, although it rose slightly on very hot days. The food, a mixture of peas, wheat and maize, was supplied in excess.

The drinking fluids were offered to each bird in a commercial "Clipper" drinking fountain formed of an inverted glass tube with a plastic trough at the base. The effect of evaporation during the relatively short periods of testing was found to be negligible. The birds were kept on a strict daily schedule, as outlined in Table I. Following overnight fluid deprivation, each bird

Table I.

Period	Time	Duration	Fluid supplied
Test	09.30 to 11.30 hr.	2 hr.	Test solution
Rest	11.30 to 13.00 hr.	1½ hr.	No fluid
Maintenance	13.00 to 17.00 hr.	4 hr.	Distilled water
Deprivation	17.00 to 9.030 hr.	16½ hr.	No fluid

was presented for two hours with only one drinking fountain which contained the test solution *or* distilled water, and the volume drunk was measured. After a rest period, when no fluid was supplied, the birds were offered a single tube of excess water during the afternoon maintenance period. During this latter time the birds were able to satisfy any fluid deficiency. Every fourth day, throughout the experiments, water was given during the morning test period and the mean volume drunk represented the reference level by which the relative preference or rejection of the test substance was measured.

The pigeons were divided into groups of eight at random and each group was tested with a

separate substance. After a period of habituation when water was given during the test period, the birds in any one group were offered the test substance at the first concentration on three successive days. On the fourth day water was given, and then another concentration of the same substance was tested for the following three days.

Results

In addition to measuring the volume of test fluid drunk, the water consumed during the afternoon maintenance period was also recorded so that the total daily fluid intake for each bird was known. Some slight daily variation in total fluid intake was noted and, as recorded above, it proved impossible to maintain the aviary at an exactly even temperature in the early afternoon during very hot weather. However, a rise in total fluid intake did not generally coincide with these small temperature fluctuations, but probably followed a period of increased activity amongst the birds which were able to hear one another flapping their wings. The results obtained are illustrated in a series of graphs (Figs. 1 to 9), but instead of correlating the mean daily intake of test fluid per bird with the concentration of the test substance, the total intake of test fluid for the group is expressed as a percentage of the overall total fluid intake. The two methods of presentation produce almost identical graphs (see Duncan, 1960), but by calculating the consumption of test fluid on this percentage basis, allowance is made for any daily fluctuation in overall thirst. Each point on the graphs therefore represents the mean value for eight birds on three days (i.e. 24 readings). The value for water as a test substance (0·0 per cent. concentration), however, is generally the mean of 72 readings. The preference or rejection shown by the pigeons for the different substances at the varying concentrations can then be related to the "water level" for each group.

The individual birds showed considerable variation in total daily fluid requirements, varying from extreme mean values of 14 to 62 cc. This variation was also reflected in the consumption of the test fluids, although each bird retained its own pattern of drinking, not only during the three days at any one concentration, but throughout the period of the experiments. A heavy drinker maintained a high level of fluid intake throughout and the mean values obtained for each group at the different concentrations are therefore unaffected. However, in testing the significance of the results, the inter-bird variation necessit-

ates the use of an analysis of variance with multiple classification. In this way, the variance between birds can be extracted.

The substances investigated were all simple and are readily classified within the four classical taste modalities of man, and are described under these headings:

1. Sour Stimuli

Substances which taste sour to man all produce hydrogen ions on dissociation in aqueous solution (see Geldard, 1953). The significant rejection of a weak organic acid, acetic acid, is shown in Fig. 1. Increasing concentration of the
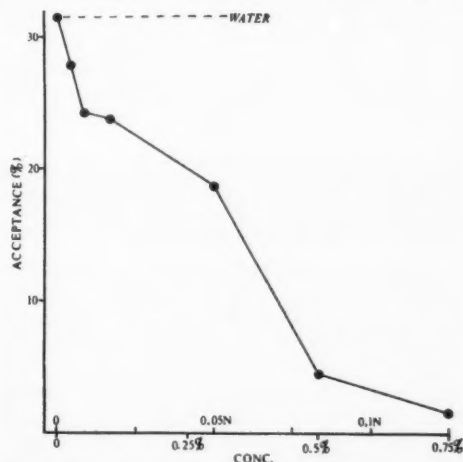
Fig. 1. Rejection of acetic acid at different concentrations. The comparison with water (0 per cent.) is emphasised. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae=concentration of test solutions.

acid produces an increased rejection; only 1·7 per cent. of the total daily drinking was done during the test period when 0·75 per cent. acetic acid was presented.

Marked rejection was also found in another group of birds which were tested with hydrochloric acid (Fig. 2). The concentrations given were relatively high for an acid which dissociates almost completely and seven of the eight birds showed almost total rejection at even the most dilute solution tested (0·075 per cent.). However, the remaining bird (No. 15) continued to drink the acid solutions at each concentration with little change from its pattern of drinking when it was offered water as the test solution (Fig. 2).
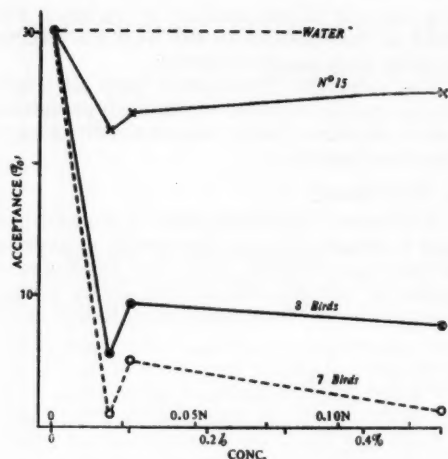
Fig. 2. Hyrdrochloric acid. Response of all eight birds in the group, of aberrant bird No. 15 and of the remaining seven bird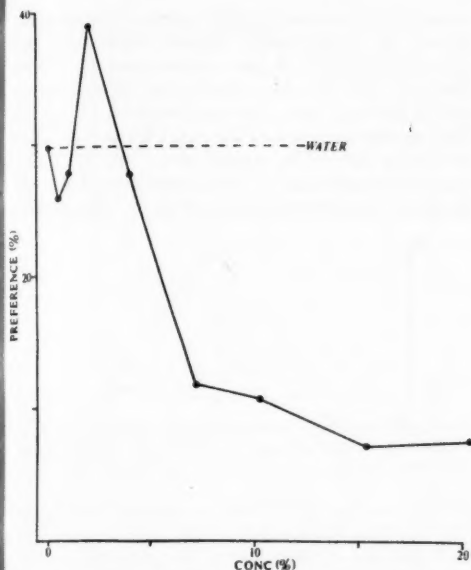s is shown. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae=concentration of test solutions.

An analysis of variance between the results obtained for water and for 0·004N (0·025 per cent.) acetic acid shows that there is a significant reduction in intake at the latter concentration (variance ratio, F, highly significant at 5 per cent. level). If this represents a genuine response to the gustatory stimulus, it suggests that pigeons are surprisingly sensitive to sour-tasting solutions, since 0·005N acetic acid only just tastes sour to man (see Moncrieff, 1944, p. 97). Analysis of the results over the whole range of concentrations tested shows, of course, that F is highly significant at 0·1 per cent. level.

2. Salt Stimuli

The response of the birds to different concentrations of sodium chloride is similar to that shown by mammals. The mean daily intake exceeds the corresponding value for water consumption at concentrations around 0·5 per cent. (Fig. 3), indicative of a marked preference. An analysis of variance (multiple classification) of the results at these lower concentrations, 0·0 per cent., 0·1 per cent., 0·25 per cent. and 0·5 per cent. shows that the rise in consumption is significant (F highly significant at 1·0 per cent. level). With solutions of 1·0 per cent., and at higher concentrations, increased rejection was found.

The results correspond closely with those ob-
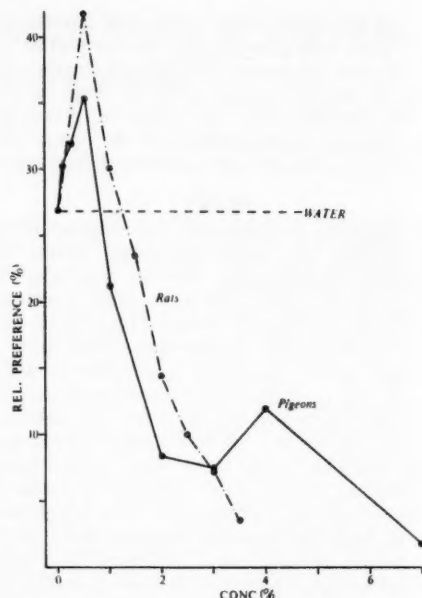
Fig. 3. Preference for sodium chloride. Comparison of response of rats and pigeons is shown. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. The ordinates for the rats are adjusted to equate the two water levels. Abscissae=percentage concentration of test solution.

tained by Weiner & Stellar (1951), using a similar experimental technique, during their study on the rat and the comparison is illustrated in Fig. 3. The data for the rat are approximate, being taken from a graph given by Weiner & Stellar, and are adjusted to equate the water level to that of the pigeons.

The pigeons were also given calcium and potassium chlorides and an essentially similar pattern of acceptance was observed (Figs. 4 and 5). In both cases, however, a small rejection (F significant at 5 per cent. level) was found at the lowest concentrations, which preceded the rise in acceptance to a definite preference at medium concentrations. Analysis of these results (0·0 per cent. to 2·5 per cent. concentrations) shows the rise in preference to be statistically significant (F highly significant at 1 per cent. level for $CaCl_2$ and at 0·1 per cent. level for KCl). Increasing concentration again produced an increased rejection.

The responses shown to the three chlorides investigated are compared in Fig. 6 with the concentrations of the solutions expressed in

Fig. 4. Calcium chloride. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae =percentage concentration of test solution.
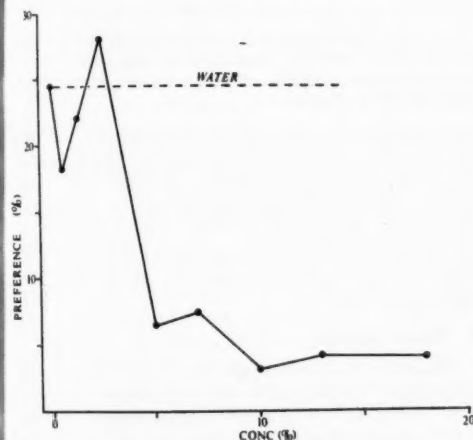

Fig. 5. Potassium chloride. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae=percentage concentration of test solution.

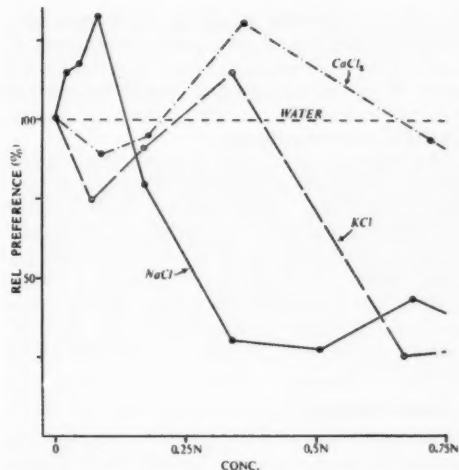terms of normality. The ordinates are adjusted to equate the respective "water levels". From this graph it can be seen that the marked preference for sodium chloride occurred at a much lower

Fig. 6. Relative preference for three chlorides. Ordinates are adjusted to equate the respective water levels, arbitrarily termed 100 per cent. Abscissae=concentration of test solution expressed in terms of normality.

concentration (0·085N) than the corresponding peaks of calcium and potassium chlorides (both about 0·35N). At the latter concentration, however, sodium chlorides is markedly rejected. Frings (1948), however, found that the stimulative efficiencies for man of the three cations as chlorides are in the reverse order, $Na^+ < Ca^{++} < K^+$, being assessed from the respective sensitivity thresholds. He obtained a similar series when investigating the "rejection thresholds" of rabbits, although with the relatively high average values of 1·4N (NaCl), 1·4N ($CaCl_2$) and 1·1N (KCl). Frings points out that this series corresponds with the ionic mobilities of the cations and suggests that stimulation of contact chemoreceptors is the result of penetration or of surface activity. Pfaffmann (1955), using electrophysiological techniques and working at suprathreshold concentrations with cats and rabbits, also found that potassium chloride was more effective than sodium chloride, whereas the stimulatory effect of these two electrolytes was reversed for the rat.

It is possible that the two lowest concentrations of potassium and calcium chlorides employed in the present investigations are around the threshold levels for pigeons, the birds detecting something in the water which is not definitely saline in taste. The average threshold at which rats first distinguish between water and solutions

of sodium chloride is about 0·05 per cent. (0·008N), (Richter & MacLean, 1939), and, as shown above, there is a broad correlation between the preferences of this species for sodium chloride and those of the pigeon at suprathreshold concentrations.

The pigeons continued to drink small quantities of solutions of all three chlorides at concentrations above 1·0N, and yet 3·0N calcium chloride, for example, is extremely unpleasant to man. Bartholomew & Cade (1958) offered house finches (*Carpodacus mexicanus*) a choice between test solutions and water, and they also report a rejection of sodium chloride, in comparison with water intake, at concentrations above 0·15N.

3. Sweet Stimuli

The responses of the pigeons to glucose and sucrose are shown in Figs. 7 and 8. With glucose there is a small rise in preference at 3·0 per cent. (F significant at 0·1 per cent. level), but in general
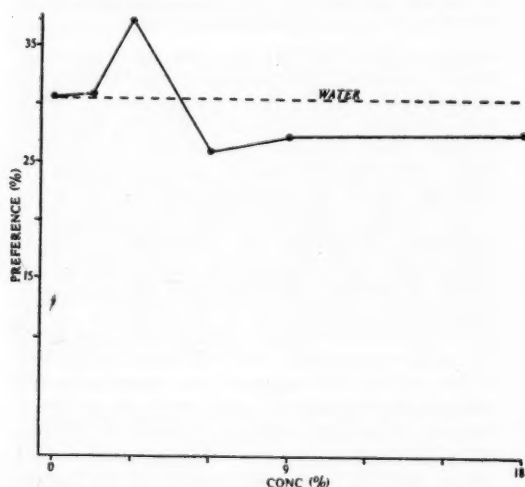
Fig. 7. Glucose. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae=percentage concentration of test solution.

there was little modification from the pattern of drinking throughout the range of concentrations tested.

A marked preference was shown for sucrose with a peak at 14·0 per cent., (analysis of all the concentrations shows F highly significant at 0·1 per cent. level). A drop in consumption was

noted at 3·0 per cent., reminiscent of the response shown by birds when offered potassium and calcium chlorides at low concentrations. Above 14·0 per cent. there is a steady fall in acceptance and at 28·0 per cent. the consumption of the test fluid shown by seven of the eight birds had fallen to almost zero. The eighth bird (No. 59) maintained a steady level of drinking throughout the range of concentrations (see Fig. 8). The decline
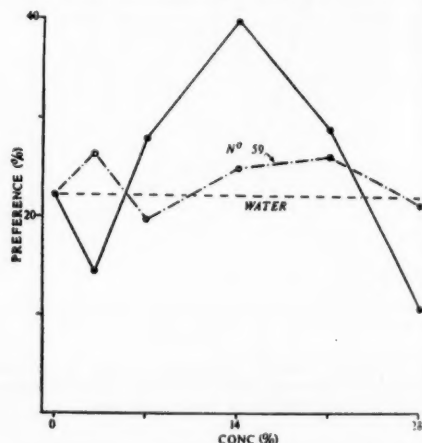
Fig. 8. Sucrose. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae= percentage concentration of test solution. Response of bird No. 59 is shown.

in acceptance at the higher concentrations shown by the other birds is not necessarily the result of gustatory stimulation alone, however, because such solutions are very viscous and as suggested previously (Engelmann, 1943; Duncan, Wright & Ridpath, 1960) tactile sensation predominates over strong taste stimulation when the bird selects its food.

4. Bitter Stimuli

Quinine hydrochloride was the only bitter substance investigated and marked rejection was noted at all concentrations tested 0·1 to 2.0 per cent.; Fig. 9).

Discussion

It was not possible during this preliminary investigation to use some birds which had previously suffered lingual denervation, but it is hoped that it will be possible to include such controls in future experiments. The results of the preference tests show, however, that feral
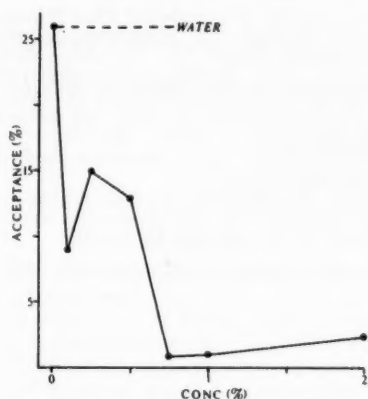
Fig. 9. Quinine hydrochloride. Ordinates=intake of test fluid expressed as percentage of the total fluid intake. Abscissae=percentage concentration of test solution.

pigeons respond to a wide range of taste stimuli, although histological examination suggests that their chemosensory equipment is limited. Elaborate training experiments would be necessary to demonstrate conclusively that birds can distinguish between different gustatory stimuli, but in general the preference-aversion responses correspond with results obtained from mammalian studies. It also seems probable that the thresholds for salt and sour stimuli are of the same order of magnitude in these two groups of animals. The stimulative efficiencies of sodium, potassium and calcium chlorides for birds, however, appear to be in the reverse order to that obtained during preference studies with the majority of mammals (see Pfaffmann, 1951). Although both hens (Kare *et al*, 1957) and pigeons respond to a wide variety of gustatory stimuli when these are added to the drinking water, taste is probably of much less importance in the slection of food, (Engelmann, 1940, 1941; Duncan *et al*, 1960; Duncan, 1960).

Of the 60 birds studied, two behaved differently in their responses from the other members of their group. No. 59 continued drinking high concentrations of sucrose, apparently undeterred by either the taste or the viscosity. As suggested previously, either of these two factors may be responsible for producing the rejection shown by the other seven birds in the group. Bird No. 15 (Fig. 2) appeared to be indifferent to hydrochloric acid and showed no marked response at the concentrations investigated. This condition of "taste blindness" may be related to the constitution of the saliva, since it has been suggested that there is a chemical interaction between a weak acid and the saliva, the latter acting as a buffer, (Pfaffmann, 1951).

Summary

The preference-rejection responses of feral pigeons have been investigated, using the method of single stimuli. The results are related to the consumption of water. The birds showed a marked rejection of sour and bitter solutions, whilst with salty substances the mammalian pattern was found, with preference at lower concentrations followed by increasing rejection when more concentrated solutions were offered. The pigeons were almost indifferent to glucose solutions, but a significant preference was found for solutions of sucrose at concentrations around 14 per cent.

Acknowledgments

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AGGRESSIVE BEHAVIOUR AND SOCIAL DOMINANCE IN THE SIX-LINED RACERUNNER *(Cnemidophorus sexlineatus)*

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Introduction

Social dominance is often observed in gregarious mammals and birds, especially those species which live in flocks, herds or colonies. However, social dominance may also occur in species where aggregations are loose associations. Various forms of social dominance are known also for fish, amphibians and reptiles (Evans, 1955).

The six-lined racerunner *(Cnemidophorus sexlineatus*—Teidae), a lizard, ranges over the southern two-thirds of the eastern United States where it lives in a variety of terrestrial habitats; occurring most abundantly in open, sparsely vegetated fields and lake beaches, but also in wooded areas with undergrowth.

Population size may be as great as 300 to 400 per acre. Individual activity ranges are generally limited to areas less than 100 feet in diameter and these ranges overlap broadly. Daily activity is greatest in late spring and early summer with a sharp decline in adult activity during late August and early September (Carpenter, 1959).

Slight sexual dimorphism is apparent, where the stripes of the male are partially obliterated by the suffusion of green laterally, and his belly takes on a bluish tinge in contrast to the prominent stripes and white belly of the female.

During the summers of 1955 and 1956 observations on a natural population suggested that aggressive behaviour in this species results in dominance relationships and a number of experiments was performed to verify these preliminary observations.

Methods and Procedure

Observations of the six-lined racerunner were made (1) upon a large natural population, (2) in enclosed experimental habitats, (3) in large cages, and (4) incidental to other field work. The natural population was located on the shore of Lake Texoma, three miles west of the University of Oklahoma Biological Station, Marshall County, Oklahoma. This area, Racerunner

Bluffs, possesses an apparently large population of *C. sexlineatus* and its topography and vegetation allowed detailed observations to be made at a distance.

Two experimental habitats were used. One of these was formed by enclosing a 15 ft. × 10 ft. area at one end of a laboratory room on the University campus at Norman, and adding sand, logs, and sparse vegetation to simulate a beach environment. The other consisted of an area 8 ft. × 5 ft. enclosed by galvanised sheet metal sides and hardware cloth ends, three feet in height. The central third of the area was covered with a mound of sand 20 inches high, while the rest was covered with Bermuda grass, one inch high. This enclosure was located at the University of Oklahoma Biological Station, on Lake Texoma.

Cages used in holding stocks of lizards, and for some observations, were glass and/or quarter-inch hardware cloth panelled units measuring 30 in. × 30 in., or a combination of four units measuring 60 in. × 60 in. × 30 in. One inch of sand was placed over the bottom of the cages.

The racerunners were captured and marked by toe clipping and by marking the dorsal shoulder or pelvic area with a bright paint for individual recognition at a distance. The lizards used for observation were obtained chiefly from large populations found on or near sandy fields of peanuts and cotton within a few miles of the Biological Station. The periods of time in captivity before use in an experiment varied from one day to a number of months.

Observations were made on the natural population with the observer, aided by field glasses, stationed at the top of the bluff. The observer at the experimental habitats sat just outside of the enclosure, while in the laboratory habitat, he was behind a blind. The lizards quickly became accustomed to the observer's presence, and it is not believed that this had any significant influence on the type of behaviour being recorded.

The time of observation was generally limited

to late morning, between 9.00 a.m. and 11.30 a.m., the period of greatest activity for the lizards as established by two summers of observations on the natural population. This period is approximately the same as that found by Barden (1942) in laboratory experiments on periodicity in this species.

The length of time a group was observed varied, depending on the degree of activity of the lizards, which was directly influenced by weather conditions. The greatest activity occurs on clear days at about 90 degrees Fahrenheit, both in inside and outside enclosures. Temperatures inside were raised by steam heat radiators, but no attempt was made to maintain a particular temperature.

Experimental Groups

The Following Groups of Lizards were Observed at The Biological Station (BS) and Behaviour Laboratory (BL) at Norman during 1957 and 1958. All were adult size except for group 6 which were small individuals.

Group	Date of observation	Length of observ. period	No. of males	No. of females	Location	No. of encounters
1	24 July-3 Aug. 1957	11 days	6	4	BS	112
2	6 Aug.-12 Aug. 1957	7 days	7	1	BS	94
3	27 Oct.-28 Dec. 1957	63 days	5	5	BL	79
4	23 June-26 July 1958	13 days	5	5	BS	208
5	7 July-13 July 1958	7 days	10	0	BS	216
6	14 July-23 July 1959	10 days	6	4	BS	51
7	24 July-4 Aug. 1958	12 days	6	4	BS	186

All of these lizards in the seven groups sunned, fed, burrowed and acted in a manner similar to those observed in the natural population.

General Observations

The six-lined racerunner moves over the ground in a series of short, jerky spurts, giving the observer the impression of alertness and nervousness. While foraging, it may climb up on weeds and low bushes, nose objects on the ground, investigate small holes and crevices and occasionally dig or scratch the ground. It digs a burrow for a retreat which extends from one to four inches under the soil, or it may excavate a shallow depression beneath a log, rock or some other similar object. These burrows or retreats are used when the lizard is not actively feeding or sunning.

Mating is most frequent in late spring and early summer, but it has been observed in late summer and, in laboratory populations, throughout the winter. A characteristic action of adult males in the presence or absence of females, but presumably associated with pre-courtship, is "cloacal rubbing," in which the pelvic region is moved rapidly from side to side on the ground, the male moving slowly forward, sometimes describing a figure eight. A male may attempt to mate with any other lizard of his species (Noble & Bradley, 1933) but on finding a receptive females, straddles her, then curves his body over hers, bites her on the flank of the side opposite to that where his cloacal region approaches to effect copulation.

Evidence of aggressive behaviour in this species can be cited from many different field experiences. While walking and driving along southern Oklahoma roads racerunners have often been observed giving chase, commonly in the form of one racerunner dashing across or down the road pursued by another.

At Racerunner Bluff, when activity was greatest, frequently as many as ten different individuals were observed within a period of one minute. When a number of individuals were in view at one time, it was apparent that they were usually scattered over the study area, no aggregations being observed. When two individuals approached close to and became aware of one another, one usually pursued the other immediately. Such encounters were frequent, on some days as many as two or three chases took place at the same time within the study area, all involving different individuals. Territoriality was not apparent as indicated by plotted movements over the area (Carpenter, 1959).

The results of these encounters and chases were variable. Most involved only quick pursuit with the pursuer halting its chase after approximately five to ten feet. Some chases continued over a distance of approximately 100 feet. Even such long pursuits may have involved no more than a few seconds. In pursuits wherein contact was made, and these frequently occurred, the pursuer caught up with the retreating individual and bit or grasped it along the base of the tail. The grip was usually broken quickly, and the chase continued or was terminated by the escape of the pursued lizard. Only rarely was a portion of the tail lost. On occasion, the grip on the tail was secure, with the result that the pursued then began to roll over and over on the sand until the grip was released, the pursued quickly retreating again. The retreating lizard, when bitten, sometimes will bite his attacker in return or may, if the bite upon it is secure, attempt to force the attacker to release with the roll, in which the attacked lizard rolls over and over causing the attacker to roll with it until it releases its grip. Chases were observed where two lizards were retreating in the same direction from one pursuing lizard. This pattern was probably due to the activity of the one retreating lizard arousing another and causing it to retreat.

The retreat of the adult female from a male sometimes varied from that of the male or juvenile. The female, in these encounters, did not retreat from a male as quickly, which may in some way (not determined) have been a response to some action of the male. In these retreats, she started quickly, then moved away in short spurts, the male following the female in a similar manner. Further study will be necessary to determine the relationship of this female retreat to sexual behaviour. Retreats may begin at distances of up to one or two feet.

When the pursued lizard was retained by the grasp of the pursuer or where the escape of the pursued was almost impossible due to the superior strength and persistent effort of the pursuer, the subordinate lizard then might assume a submissive posture. The submissive lizard flattened out on the ground, head pressed to it, legs spread wide, eyes closed, and maintained this position without further response until the aggressor left. Some individuals remained in the submissive posture for as long as five minutes after the molesting ceased. A few observations were recorded where the submissive individual rolled over and remained on its back.

The six-lined racerunners placed in cages up to 60 in. $\times$ 60 in. $\times$ 30 in. in size showed little or none of the type of aggressive behaviour here discussed, that is, chases did not occur. Perhaps for dominance to be expressed through aggressive behaviour, these lizards must have a certain amount of space available. This may be related to the "individual distance" Hediger (1950).

At times, when two or more racerunners were being held temporarily in a gallon jar while marking them for release, they were observed to bite one another; one holding onto another by the head, neck or tail for as long as three to four minutes. Racerunners taken from funnel traps, when more than one was present, frequently showed stubs of recently broken tails, the tail pieces being present in the trap. No explanation other than being broken in a fight with another lizard could be found. In one instance it appeared that a lizard had been killed by one of the other two lizards present in the trap.

Neutral encounters occurred when two lizards on becoming aware of one another both retreated in opposite directions. Such encounters were not frequent, and some that did occur could be explained in the following manner. In vegetation which was high enough to impair or occlude vision at short distances, two lizards would frequently be as close as one or two inches before they became aware of one another's presence. In these circumstances, both often fled in opposite directions, probably before individual recognition was made.

Dominance Behaviour in Experimental Groups

Dominance behaviour occurred when racerunners encountered one another and one retreated (subordinate) from the other (dominant), pursuit frequently taking place. An individual was designated as dominant for that group when it won the majority of encounters with the greatest number of individuals within its group, and similarly as subordinate when it lost the majority of its encounters.

The following criteria were used to determine the social rank for each individual of each group; number of individuals dominanted, number of individuals subordinate to, number of encounters won with respect to the total number of encounters. The higher the rank, the greater the number of individuals dominated, the fewer subordinate to, and the greater the total number of encounters and the percentage of encounters won. The middle ranks were difficult to determine and were frequently arbitrary, and might be thought of as having equal rank,
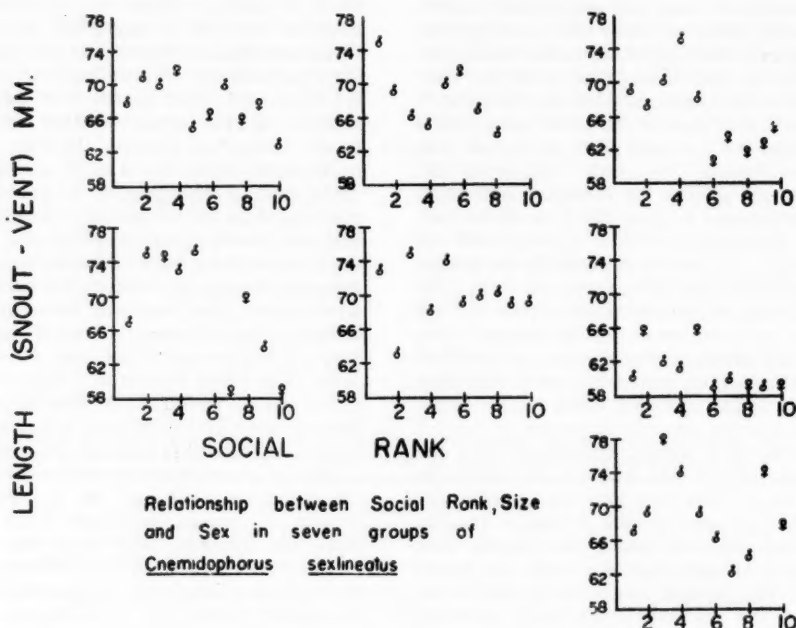
LENGTH (SNOUT - VENT) MM.

SOCIAL   RANK

Relationship between Social Rank, Size and Sex in seven groups of Cnemidophorus sexlineatus

Fig. I

The presence of dominant and subordinate individuals in the seven groups tested was strongly evident. In each group there were one or two individuals that were dominant over all other members of the group. Also present were one or more individuals which were strongly subordinate. Between these extremes of dominant and subordinate positions in the groups were individuals indicating dominance towards certain members of their respective groups and subordination with respect to others.

The most dominant individual of each group except one was involved in the greatest number of encounters for its respective group. For all groups, the most dominant lizard was involved in an average of 49·3 per cent. (range 27 to 63 per cent.) of all encounters. In some groups, encounters between particular members were never observed. More encounters were recorded between males proportionately than between females or males and females.

The largest lizard (body length) was the most dominant individual in only one group; in the other groups, the dominant varied from third to seventh in size within their respective groups.

However, it was generally true for all groups that the larger males averaged higher in rank than the smaller males. The smaller lizards of all groups were usually subordinate. Size, then, was not necessarily a factor in social dominance (Fig. 1).

Females appeared to be generally subordinate to males. When females did win the majority of their encounters with a male, this male (or males) was usually a smaller or the smallest subordinate male(s) (Fig. 1).

Among only males, the most subordinate male was the smallest in five of the seven groups, while in the other two, he was one of the smaller males.

Among only females (in the four groups where more than one female was present and where female-female encounters were observed) the largest female was most dominant in three groups, while in the other group, it was the second largest female that was dominant.

Observations on the group usually started before the lizards became active and were continued until activity had generally ceased. The dominant lizard of each group was usually the

first lizard to become active in the morning and it was also active over a longer period of time during the day. For this reason they may also have been engaged in the greatest number of encounters. The smaller lizards showed less activity than the larger individuals which probably accounts for their involvement in fewer encounters. Further evidence of less activity in the smaller lizards was indicated in Group 6 where all were small lizards and only 53 encounters were recorded.

Towards the end of a morning's period of observation, subordinate lizards appeared to be more alert and excitable perhaps as a result of the persistent aggressive behaviour of the dominant lizards.

While Group 5 was being observed, a test was run to see if the lizards had become conditioned to the white paint mark of the dominant. An active individual was given a similar mark. No change in the social dominance pattern was observed. From this it was thought that the paint marks used had no effect on dominance, or that the members of the group did not become conditioned to the paint marks.

Towards the end of the observations on Group 4, the dominant was removed from the enclosure. There then appeared to be an increase in the activity of the other members of the group with a new dominant appearing.

Three of the males used in Group 7 were the dominants from each of Groups 4, 5 and 6. The dominant from Group 4 turned out to be also the dominant in Group 7, while the other two dominant individuals from Groups 5 and 6 held ranks four and seven respectively. The dominant of Group 6 was a small individual and from the general results of all groups could be expected to hold this lower rank when placed with larger individuals.

Discussion

Aggressive behaviour is characteristic of vertebrates in general and the function of this type of behaviour is variable. Individual aggression (Collias, 1944) may be expressed in two types (*a*) defence of a given area (territory) and (*b*) hierarchies of precedence within social groups. The aggressive behaviour of the six-lined racerunner tends to be of the latter type.

The aggressive behaviour of the six-lined racerunner is characterised by chasing and frequent associated biting, as observed in both natural and confined populations. In the seven groups of these lizards observed, certain individuals in each group were more aggressive and dominated others of the group, and when a social rank arrangement was attempted, a hierarchical system of organization was apparent. The higher and lower ranks were readily distinguishable, while the intermediate ranks were less recognizable.

Limited activity ranges have been described for the six-lined racerunner (Fitch, 1958; Carpenter, 1959) and also for other species of *Cnemidophorus* (Milstead, 1957), but none of these workers has observed behaviour indicating territoriality as characterised by certain iguanid lizards (Greenberg & Noble, 1944; Fitch, 1940). These iguanid lizards have developed elaborate behavioural displays used in declaring territory. No such display is known for the six-lined racerunner, though some sexual dimorphism in colour is present.

Aggressive behaviour may be associated with sex recognition and mating. The varied response of the female to the dominant male suggests that sex recognition is possible and that pursuit and biting may be attempts to subdue and mate with an individual. However, in courtship, the male straddles a receptive female just before grasping her flank and proceeding with copulation. No such straddling was observed in the aggressive actions in this study. Noble & Bradley (1933) working with this species in small cages, record large males in homosexual mating attempts. Since only adult females were observed to respond differently when retreating from adult males, the homosexual behaviour observed by these authors may have been partly due to the small confined area for observation (glass-sided, screen-topped cages, 62·5 cm. long, 40 cm. wide, and 47·5 cm. high). However, the response of subdued subordinates in enclosed groups, in which these usually small lizards assumed the above described submissive posture, may indicate that homosexual mating attempts may be made in nature, the submissive posture and the rolling being a defence against this type of mating and a means of sex recognition. This is further supported by observations where a large dominant male on a few occasions was observed to perform "cloacal rubbing", a sign of sexual excitement, while it had an overpowered subordinate in a submissive response.

Since territoriality is apparently not developed in *Cnemidophorus*, the aggression and dominance established by intraspecies conflict could function in regulating population density as would territoriality in species showing this behaviour. The dominant individuals assuring an

adequate food supply for themselves by driving off their subordinates to less favourable habitats.

Summary

Field and experimental studies on the lizard *Cnemidorphorus sexlineatus* (six-lined racerunner) were made on marked populations in Oklahoma. Aggressive chases, followed often by biting, were common in both field and confined populations. As a means of testing dominance, as indicated by aggressive activity, seven groups of racerunners were confined in simulated natural habitats both indoors and outdoors.

In any particular encounter between two individual lizards, the pursuer was recorded as dominant and the pursued as subordinate in social rank.

Each group had one or two individuals which were more aggressive and accounted for over one half of the recorded encounters. Males were usually dominant over females, and the smallest lizards were the most subordinate. Dominant lizards were more active over longer periods of time. A peculiar submissive posture was observed occasionally in subordinates. The retreat behaviour of the female was sometimes different from that of the male.

In the field, aggressive behaviour could not be directly related to possible territoriality. Courtship display was not apparent for this species and the chase and retreat behaviour may be a necessary part of sex recognition and attraction.

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THE FORAGING METHOD OF PHARAOH'S ANT, *Monomorium*
pharaonis (L.)

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Introduction

Monomorium pharaonis is a widely distributed ant which has been studied in Scotland by Peacock and his collaborators (Peacock, 1950; Peacock & Baxter, 1950) where it was infesting heated buildings. In Western Nigeria it is the commonest house ant and nests principally in the structure of houses. A small number of scout ants hunt independently for food, which consists of dead insects, scraps of human food and drops of water. When one of them finds food it returns to the nest and almost immediately a number of ants comes out of the nest and runs to the food. In less than 30 minutes a large foraging population develops, and consists of a cluster of several hundred ants feeding at the food and one or more lines of ants passing in both directions between the food and the nest. Ants returning to the nest have a full crop or are carrying small pieces of food. They always walk head foremost and never walk backwards to drag food either singly or in groups. Three main problems arise in such a foraging method:

1. how scouts are able to find their way to and from the nest;
2. how they recruit workers to foraging acttivities;
3. how they guide recruits to the food they discover.

It will be shown that these are met by a combination of two types of communication. The first type, the ability of scouts to activate other workers in the nest has been demonstrated for *M. pharaonis* by Sudd (1957b), and its effects in the field are now reported. The second type, which depends on the ability of *M. pharaonis* workers to lay and follow scent trails, is shown below to be important in the orientation of both scouts and recruits.

Methods

Monomorium pharaonis was convenient for experiment because many houses in Ibadan were infested and trails were formed freely across floors by day. Scout ants were studied by tracing

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the paths they followed on their way to or from the nest. They were relatively insensitive to close observation and it was possible to draw the track of an ant directly behind it in pencil provided that the pencil was not brought nearer to the ant than half an inch. In investigations of the return of the scouts to the nest ants could be selected from those leaving the nest as scouts and placed on a food bait with a camel-hair brush. In most cases these ants fed and then returned to the nest. Baits such as jam were used in these experiments and ants which had fed could be told by their swollen and translucent gasters; only fed ants were used in experiments. The arrival of recruits was studied by laying baits in areas patrolled by scout ants; the number of recruits arriving at the bait or leaving it in each half-minute was recorded. Dead insects, scraps of meat, jam, sugar solutions and water, exposed on a $\frac{7}{8}$-inch circular cover-glass, were used as baits.

Results

1. Behaviour of Scout Ants Searching for Food

Most colonies became inactive during the night and foraging began again between 0700 and 0900 hours. Scouts left the nest and began to search for food independently of each other. Their search was not, however, random since they left the nest only by well-defined routes whose position changed only slowly, over periods of some weeks. As a result the distribution of scouts around the nest was uneven. An area round the entrance of one nest was marked with 20 8-inch squares and the number of ants in each square recorded at intervals. The total number of scouts seen was 40, which was too small to allow analysis square by square. Application of a χ^2 test to the four rows of squares however gave $\chi^2 = 34.4$ (P < 0.001) a highly significant result. The same test applied to the five columns yielded $\chi^2 = 8.25$ (P > 0.05); this lack of significance was due to the course of a trunk route which ran along a row but cut across three columns. Not more than four such trunk routes were found at one colony, and 2 or 3 were more usual. The routes were determined partly by

topography; a route from a nest between a door-post, and the wall for instance reached the floor by following the edge of the door-post, and minor features such as small cracks seemed to be followed in other cases. On the other hand routes always led to areas where food was likely to be found. The same colony had another route which led across a wall to a window-sill where dead insects were found. Trunk routes sometimes ran for relatively large distances over walls or floors before an area rich in food was reached. The longest route measured was 31 feet long, and such distances were not uncommon. This agrees with the observations of Peacock & Baxter (1949) who found trails up to 40 feet long in Scotland.

Where a trunk route reached an area where food was found it broke up into smaller branches. When the tracks of scouts were marked at such places branches could be recognized where one ant followed a course that another had taken some time previously. Ants did not conform as closely to branch routes as they did, nearer to the nest, to trunk routes, and they frequently wandered away from branch routes. Ants which left a branch route in this way often returned to it after travelling a few centimetres, but sometimes joined other routes or continued to wander independently of any route. Ants left the route at some points more frequently than at others, and ants which did not leave the route at such points often hesitated there. Because of the low density of scouts on routes no statistical analysis of this was possible; to group the data would conceal the existence of the points at which ants left the route and observations made on different parts of the route cannot easily be compared. However, in three portions of route only 6 out of 70 ants observed left the route at independent points. Of the remaining 64, 9 left at each of 2 points, 6 at 3 points, 5 at 4 points and 4 at 2 points. The total number of points was 17 over a length of 36 cm., so that the mean distance between points was 2·1 cm. or about 7 times the length of a worker ant. Some of these points were later found to be points of branching, but at others the tracks of ants which had left the route differed.

Branch routes originated as scent trails laid by successful scouts on their way to the nest after finding food. When ants had been seen feeding in a place for the first time, the following day numbers of scouts were found there. When a source of food to which a trail had been formed was exhausted or removed, many of the ants on the trail took to scouting. In an area in which the route system had been mapped a scout was allowed to find food and return to the nest. The route by which it returned was marked and the food removed. The scout did not follow a branch route with complete accuracy, but its route was retraced with few divergences by all subsequent scouts in that part of the system and a high proportion of them remained on the route till its end. The newly formed branch route was found next day to have been incorporated in the route system. Branch routes, if formed in this way, provided a method by which the route system was adapted to the changing distribution of food. *Formica* (a species of the *rufa* group) (Holt, 1955), *Myrmica ruginodis* (Brian, 1955) and *Oecophylla longinoda* (Way, 1954) also forage on well-defined routes, but these seem to be more permanent since they lead to trees on which the ants milk Homoptera.

In experiments ants were found to be reluctant to walk on clean sheets of paper which bore no scent marks. For the same reason scouts did not travel far from branch routes so that the route system was extended only slowly. The combination of scent-laying and reluctance to walk on an unscented surface occurs also in *Eciton hamatum* (Schneirla, 1933; 1938; 1956). In this Army-ant members of the pushing party at the head of each raiding column advance short distances beyond the ground on which scent has already been laid and then turn back and are overtaken by other members. In *E. hamatum*, however, scent is laid by each ant during its advance even if no food has been found. Because of the number of ants in a pushing party at one time the head of the column advances rapidly. The trail systems of *Monomorium pharaonis* are extended slowly by a small number of scouts, acting at different times, and extension occurs only where food has been found.

2. The Scout's Return to the Nest

When a scout had fed it returned to the nest. The path by which it returned bore no relation to the path by which it arrived and was usually more direct. The scout almost always joined a trunk route or an important branch for the last part of the journey. Returning scouts have, however, been seen to cross such routes, or even to cross active foraging trails, without turning along them.

At first it appeared that the scout found its way back to the nest by following scent clues similar to those which guided outgoing scouts. However, when baits were laid on fresh sheets of
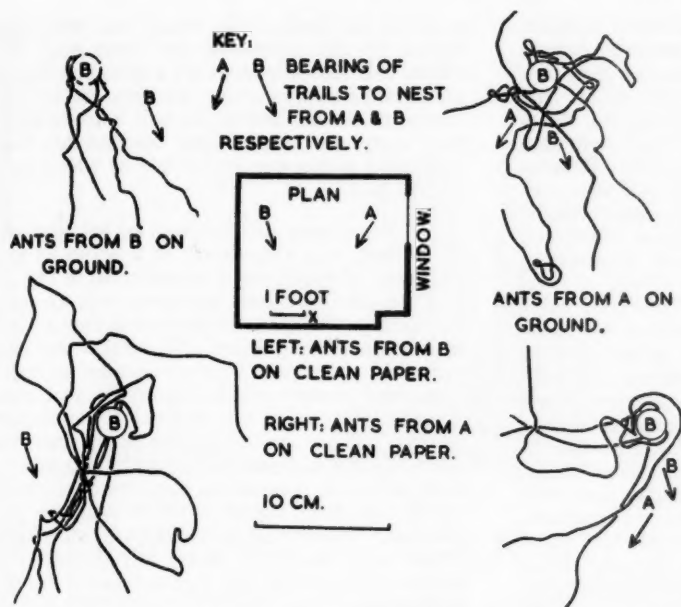
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KEY:
A ⟷ B BEARING OF TRAILS TO NEST FROM A & B RESPECTIVELY.

ANTS FROM B ON GROUND.

PLAN

I FOOT

WINDOW.

LEFT: ANTS FROM B ON CLEAN PAPER.

RIGHT: ANTS FROM A ON CLEAN PAPER.

10 CM.

ANTS FROM A ON GROUND.

Fig. 1. The interaction of an orientation to scent marks on the ground with another orientation in the homing of scout ants. The plan shows the room where the experiments were carried out. Outgoing scouts collected at B and placed on food at B were better able to return to the nest when placed on the bare floor than when placed on a sheet of paper. Ants collected at A and fed at B on a fresh sheet of paper left B in a direction more or less parallel to that which would have brought them to the nest from A. Ants collected at A and fed at B on the bare floor were confused in their orientation. Tracings made in the field have been superimposed.

paper a scout which found the bait was able to find its way back to the nest without retracing its own tracks. Other experiments had already suggested that ants could orientate to visual clues and it seemed possible that scouts might use such an orientation in returning to the nest. Fig. 1 summarizes experiments designed to test this. The experiments were carried out in a cement floored room. A trunk route reached the floor at X and branch routes radiated out from that point over the floor. At the time of the experiments no routes reached the floor except through X; later, however, branches from other trunk routes reached the floor and experiments had to cease. No other route system was found in which trails radiated over a large angle from only one point, and it was not possible to repeat the experiments since it was only in a system of this form that there could be any certainty as to the approximate direction in which ants travelled to and from the nest. Outgoing scouts were

taken from the two points A and B, and placed on a bait B. Only one ant at a time was allowed to feed at the bait and scouts which reached the bait fortuitously were removed and killed. If the ant fed at the bait so that the membranes between its abdominal sclerites were visible as pale bands, its track on leaving the bait was marked until it was about 1 foot from the bait. The ant was then killed so that no recruitment took place. In Fig. 1 the tracks of these ants have been superimposed and grouped in four classes:

1. Ants taken from B and placed on the cement floor at B.
2. Ants taken from A and placed on the cement floor at B.
3. Ants taken from B and placed on a fresh sheet of paper at B.
4. Ants taken from A and placed on a sheet of fresh paper at A.

The best homing performance was clearly that of ants in the first of these classes. Ants from A were able to home quite well when allowed to run on the cement floor but some of them made abnormally convoluted tracks and one after beginning well, turned and went in the diametrically wrong direction. Ants from B running on fresh paper were able to leave the bait in approximately the right direction though some orientated uncertainly. None of the ants from A, however, ran in the true direction of X when placed on a fresh sheet of paper and all ants in this class had a bias towards the left of the figure, that is to the course which they would have taken to reach X from A.

These results suggest that scouts were orientating to the nest by the collation of two sets of sensory clues, one from marks on the ground, and one from a distant source, probably visual. When marks on the ground were lacking (as when baits were laid on fresh paper) returning scouts depended on distant stimuli. If they had

been brought to the bait from another position, the course which they set appeared to depend on the direction they had followed on their outward journey from the nest, and would not lead them to the nest from their new position. When ground marks and visual clues were contradictory (as when ants were brought from a distance to baits on the cement floor) the ants were confused but reached the nest by rather twisted courses. The relative importance of visual and chemical clues in ant orientation is discussed by McGregor (1948) and Vowles (1955). Vowles (1955) found that workers of *Myrmica rubra* in a strange environment orientated by visual clues, including the plane of polarization of light. McGregor had already shown, however, that the same species gradually built up a system of scent marks on the ground and orientated to them. Goetsch (1934) showed that a number of species of ant which followed scent trails, simultaneously orientated visually, and that *Cremastogaster scutellaris* could, in addition, orientate to the direction of gravity. McGregor (1948) concluded that "the cause of complication in ant behaviour is to be sought in redundancy of senses; the ant can perform the same act equally well by the use of one sense or of a number of senses." *Monomorium pharaonis* apparently used a visual orientation together with an orientation to scent marks. The visual orientation requires further investigation in the laboratory, since as Vowles (1955) remarks, the analysis of light-compass reactions in the field is complicated by the many sources of visual stimulation which are available there.

3. The Recruitment of Workers to Foraging Activity

Shortly after a scout had entered the nest on its return from the food, a group of workers emerged and went to the bait. These workers fed at the bait and then returned to the nest. Their return was followed by the appearance of further workers who fed at the bait in the same way. Recruitment is thus progressive. Occasionally a queen appeared at the bait as was found in the laboratory experiments of Sudd (1957b). Fig. 2 shows the rate of arrival and departure of ants at a large dead insect. After a sharp rise to 25, due to early recruitment, the population at the bait rose only slowly and no ants left the bait. After another 12 minutes when the population was 58, the bait was cut in two with scissors, so that foragers, which had apparently been unable to penetrate the hard cuticle of the bait were able

to get at the flesh inside. Many ants were disturbed by this operation but these soon returned and fed. After that the population began to rise and increased to over 200 ants, and at least that number were still at the bait 5 hours later. It is important to note that this increase was associated with a rise in the rate at which ants left the bait.

The importance of the return of fed workers to the nest was also shown in a series of experiments of which one is summarized in Fig. 3. In this experiment only one scout was allowed to return to the nest, another scout and all the first recruits being killed. No further recruits came and soon there were no ants at the bait. Then one worker which found the bait was allowed to return to the nest and a population of over 100 ants soon built up at the bait. In these experiments ants were picked up before being killed, to prevent the blocking of the trail by the response shown to their dead by *M. pharaonis* workers (Sudd, 1957a). It is clear that a trail alone does not attract recruits unless a portion of the colony has been aroused to foraging activities.

It might be thought from Figs. 2 and 3, that the rate of arrival of recruits at a bait depended only on the rate at which fed workers were returning to the nest during the preceding minute or so. This was not the case except in the earliest stages of exploitation of a source of food. The return of workers to the nest eventually led to a large number of workers be coming engaged in foraging, and unless sufficient ants were killed to exterminate this aroused population, the number of ants foraging remained high, even if no fed workers were returning to the nest. In one experiment a bait at which over 70 workers had arrived was removed and the trails leading to it destroyed by washing. About 50 ants could be seen outside the nest at any time during the next 50 minutes although before the experiment only 16 scouts were seen in three minutes continuous observation. After 50 minutes a new bait was laid about 20 cm. from the site of the first. Fig. 4 shows that the rate of arrival of recruits at this bait was about twice that at the first bait. Similar effects have been observed with baits laid down after the removal of natural trails, and in experiments where interference with the trail prevented recruits from reaching food, a rapid increase was always noted when a trail was eventually formed.
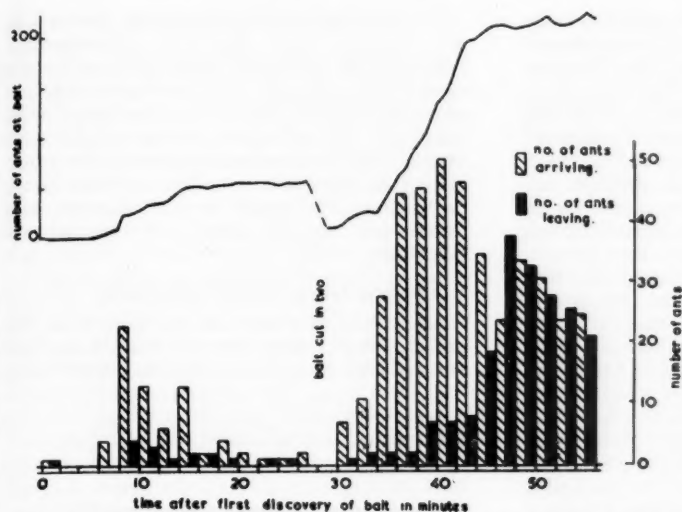
Fig. 2. The recruitment of workers to a bait which had a hard exoskeleton. Few ants returned to the nest and few new recruits came, until the bait was cut.

4. The Attraction of Workers to a Particular Bait

Field observation showed that the effect of recruitment was not merely to increase the number of foragers, and so to increase the chance that they would find the food by independent search, but was specific to the bait or to its position. When two baits lay about 5 cm. apart the first was found and attracted 40 workers, the other was not found. As the baits were identical and laid at the same time one could not have been attractive and the other not, and it is inconceivable that all of 40 ants searching independently should find one bait and none of them the other.

The paths of all ants coming to or going from a bait lay within a band 5 mm. wide and the course of this trail changed only slowly. These observations, however, apply to established trails; it does not follow that the earliest recruits find the food by following a trail laid by the scout. For instance the single trail used by later recruits might emerge as the most central of a complex of earlier trails, as Schneirla (1933) describes in *Eciton hamatum*. The path of the scout from the bait to the nest was therefore traced on the floor and the paths by which recruits subsequently reached the bait observed. In every case recruits arrived by a path identical with that by which the scout had returned to the nest. Since the scout did not return by the shortest route this could hardly be due to chance.

In other experiments which resembled those of Goetsch (1934) with *Solenopsis geminata*, a
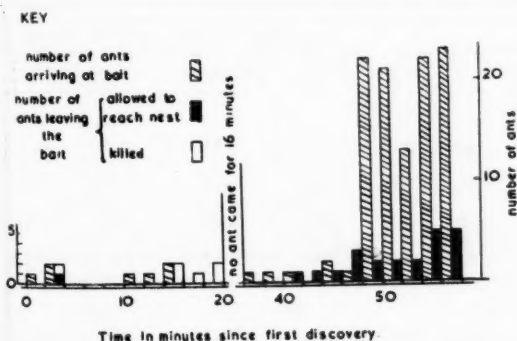
Fig. 3. The effect on recruitment of killing all ants arriving at the bait except the first, and, later, normal recruitment when ants were allowed to return to the nest after feeding.
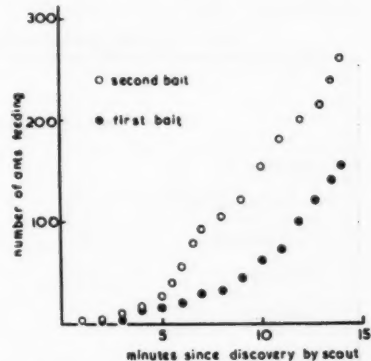
Fig. 4. Comparing the rate of recruitment to a first bait with the rate of recruitment to a second, similar bait, 50 minutes after the first had been removed and the trail leading to it washed away.

bait was laid on a sheet of fresh paper and the path of the first scout to return to the nest drawn on the paper. Any other scouts were then removed and killed. A small rectangle bearing a section of the scout's path was cut from the paper and replaced turned through an angle of 30-45°, the ends being matched carefully. Fig. 5 shows a plan of one experiment. Recruits ran along the path A and were not disturbed by errors in matching at the cut but turned through the angle by which the paper had been turned. In one experiment some recruits found their way to the bait from Z, but subsequent recruits did not follow this new path. When one ant which had reached the bait in this way had fed, it left the bait by the path of the original scout and not by the route by which it had itself arrived. When it reached the cut in the paper it hesitated, but regained trail A independently and followed it to the nest. All subsequent recruits followed the path B of this ant to and from the nest and B became the foraging trail. It was clear therefore that recruits were guided to food by trails, presumably of scent, and that these trails were laid by ants returning to the nest. It also appeared that trails were laid not by a specialized group of scout ants but by all successful foragers. Changes which occurred in persistent trails could only be explained if scent was continually being renewed by fed recruits. In one case a trail on a wall slipped slowly down the wall over the course of a few days, apparently because the gasters of fed ants hung down so that new scent was laid below the trail they were following with their antennae.

An important difference between the trails of *M. pharaonis* and those of *Acanthomyops fuliginosus* described by Carthy (1951) is that the scent laid by a *M. pharaonis* on one journey was sufficient to guide other ants to the food. Carthy found that an *A. fuliginosus* worker did not lay its trail "until the second or third run and then often incompletely." Attempts to demonstrate the scent marks made by *M. pharaonis* with lycopodium powder (Carthy, 1951) were unsuccessful.

5. The Selection of Trails by Workers

As nests of *Monomorium pharaonis* often had trails leading to more than one bait the question arose of how the proportion of the colony forag-
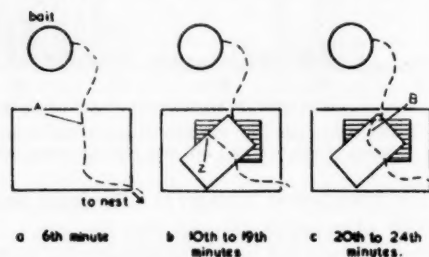
Fig. 5. *a*. The track of the first scout to leave a bait over a sheet of clean paper.

b. The paper cut so as to deform the trail laid by the scout. All recruits followed the deformed track to Z where they became lost.

c. The track of a recruit which returned to the nest after finding the bait independently. This track became the foraging trail.

Table I. The Distribution of Ants Between Two Trails and the Effect of Altering Bait Conditions.

	No. at A	No. at B	Total	Proportion at A	at B	Significance of difference from equality S.E.	Difference	"*t*"	P
Experiment 1									
1. Both baits exposed	35	41	76	0·46	0·54	·057	0·04	0·7	>40%
2. A covered	31	51	82	0·38	0·62	·055	0·12	2·2	<5%
3 B covered	47	35	82	0·57	0·43	·055	0·07	1·3	>10%
4. Both covered	51	31	82	0·62	0·38	·055	0·12	2·2	<5%
Experiment 2									
1. Both exposed	55	62	117	0·47	0·53	·046	0·03	0·65	>40%
2. A covered	68	55	123	0·55	0·45	·045	0·05	1·1	>20%
3. Both covered	52	55	107	0·48	0·52	·049	0·02	0·41	>60%

ing on each of the trails was controlled, if it was controlled at all. This was investigated in a series of experiments in which two trails were so arranged that their junction could be watched. The establishment of a Y-shaped trail had to be contrived by moving the baits after their discovery so that two trails were formed which joined at a convenient place. Any unwanted trails were eliminated. When both branches of the trail were well established the numbers of ants running on each did not differ significantly (Table I), although during the early development of the second branch there were fewer ants on it than on the first branch. The equal distribution of ants between the branches suggests that the branch which an ant followed was determined by chance, but this would need testing with greater qualitative differences between the baits. After the removal of one bait the proportion of ants on the trail leading to its former position decreased, but the change was slow so that a few ants continued to run on it for an hour or more. Significant differences in distribution were not found during the period of observation (about 30 minutes) although when one bait was replaced and the other removed a significant difference was once obtained (Table II). In Experiment 1, however, some differences produced by covering baits were significant, whilst in Experiment 2 no significant differences were found although a larger number of ants was observed. This was probably due to the more rapid arrival of ants at the bait in Experiment 2, so that although more ants were observed the period of observation was shorter. It seemed that

the lapse of time was of more importance than the number of ants which had experienced the altered conditions. Fading of the trail, due to evaporation of the scent and to the ants' failure to renew it may therefore be the cause of abandonment. This was supported in an experiment in which it was found that the proportion of ants still running on a baitless trail fell off more rapidly if the trail had been washed with a detergent solution (Fig. 6).
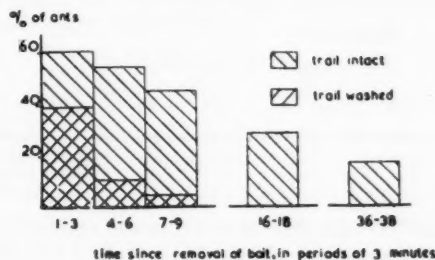
Fig. 6. The proportion of ants on a two-branched trail system that ran on the branch from which the bait had been removed (a) when the trail was intact and (b) when the branch had been washed. In both cases the other branch in the system was untouched and still led to a bait.

6. The Unimportance of the Position of the Food on the Trail

As recruits followed scent marks on the ground it seemed possible that the position of the food might also be marked in some way, perhaps as food is "scented" by the honeybee (Kalmus & Ribbands, 1952). When the bait was

Table II. Comparison of Distribution of Ants Between Two Trails Under Different Bait Conditions. (Data from Table I)

Treatment compared	Difference	S.E. of difference	"t"	P
Experiment 1				
1 & 2	0·082	0·078	1·1	>20%
2 & 3	0·195	0·077	2·54	1%-2%*
3 & 4	0·049	0·077	0·63	>50%
1 & 4	0·160	0·078	2·05	<5%*
Experiment 2				
1 & 2	0·08	0·063	1·28	20%
2 & 3	0·07	0·066	1·06	>20%
1 & 3	0·01	0·067	0·15	>80%

*Significant difference.

Table III. The Effect on the Proportion of Ants at a Far and at a Near Position on a Trail of Laying Bait at one or both Positions.

Position of bait	Ants at far position		Ants at near positions		Total	Significance of difference from equality		
	No.	Propn.	No.	Propn.		Diff. & S.E.	"t"	P
Far only*	40	1·00	0	0·00	40	—	—	—
Near only	3	0·07	38	0·93	41	0·43 ± 0·078	5·5	< 1%‡
No bait	4	0·67	2	0·33	6	0·17 ± 0·210	0·83	>40%
Both baited	12	0·34	23	0·66	35	0·16 ± 0·090	1·9	>10%
Both baited †	2	0·14	12	0·86	14	0·36 ± 0·140	2·8	< 2%‡
Far only	14	0·94	1	0·06	15	0·44 ± 0·130	3·3	< 1%‡

*For establishment of trail only.
†After renewal of bait.
‡Significant difference.

removed from the end of a trail and replaced by a similar bait 10 cm. nearer to the nest and just touching the side of the trail the new bait was accepted by nearly all ants (Table III), and those that passed it appeared to have missed it by following the wrong side of the trail. When this bait was also removed numbers at the two sites fell off and no significant result was obtained. When both baits were replaced significantly more than half the ants stopped at the first bait they encountered, that is the nearer one. Experiments were then stopped because it was clear that ants followed the trail until they encountered food and that which bait they took did not depend on the exercise of choice. Near the baits the trail broke into strands, and ants might leave the main trail along a strand and search for food at its end. This sometimes gave the impression that the site of the bait was marked. The strands arose when fed workers left the bait at various points on its circumference and joined the trail a short distance from the bait. It also appeared from these experiments that the distance of the bait from the nest was not communicated to recruits by the scout.

7. The Unimportance of the Scent or Flavour of the Bait to Recruits

When the bait was changed after it had been found by a scout but before the arrival of recruits the latter did not search for a bait resembling that found by the scout and recruitment to the substituted bait was normal (Fig. 7). This may be related to the finding of Sudd (1957b) that scouts do not regurgitate food to

recruits. Eidmann (1927) found that recruits of *Myrmica laevinodis* also were not affected by the substitution of a bait different from that on which the scout had fed, but that after they had themselves fed on it they became habituated to it and could not be deflected to another bait.
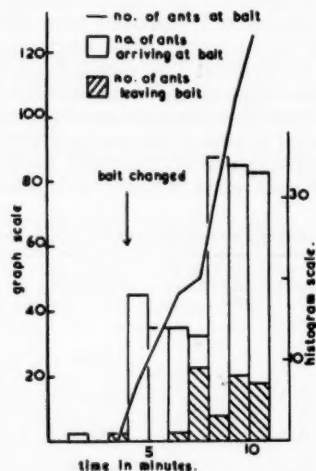
Fig. 7. Normal recruitment when a bait of jam was substituted, before the arrival of recruits, for the meat discovered by the scout.

When *Monomorium pharaonis* workers were allowed to feed on jam for two hours, and then the jam removed and replaced by two baits, one of jam and one of meat, they showed no signifi-

cant preference (Table IV). Although there was at first a bias in favour of jam this was accounted for by the more favourable position of that bait on the trail. The proportion of ants at each bait could be altered by small adjustments of the positions of the baits.

Table IV. The Proportion of Ants at Each of Two Baits (one jam, one meat) after Two Hours Training to Jam.

Total number of ants after successive ½-mins.	Proportion at jam	Proportion at meat	Range of proportions for which P 5%*
8	% 50	% 50	% 6—94
8	50	50	6—94
18	67	33	25—75
20	70	30	26—74
28	64	36	30—70
29	69	31	30—70
37	62	38	35—65
60	55	45	38—62
61	51	49	39—61

*P is the probability that proportions within the range stated would occur if ants showed no preference for one bait, P was calculated from the value of "t" for the total number of ants indicated in Column 1.

Summary

1. Foraging workers of *Monomorium pharaonis* orientated to scent marks on the ground. The scent marks were laid by other workers when they were returning to the nest after finding food. Such successful foragers orientated to the nest partly by scent marks and partly by another orientation, probably a visual one.

2. When a successful forager arrived in the nest it activated a small group of workers. These recruits followed a trail of scent laid by the scout, fed and returned to the nest by the same trail, reinforcing it with new scent. In the nest they activated more recruits.

3. Recruits were not informed by the scout of the nature of the food to which they were recruited, nor of its distance from the nest. There was no evidence that recruits became habituated to a particular kind of bait after feeding on it for 1-2 hours.

4. When recruits were running on a system with two branch trails, the branch which they took appeared to be decided by chance. The abandonment of a trail which no longer led to
food was probably due to the fading of the scent in the absence of successful foragers to reinforce it.

5. The vestiges of the foraging trails formed a system of 1-4 trunk routes leading from the nest. One of these routes formed the proximal part of every scout's return path; hence this part of the route was continually reinforced with new scent and was constant in position. All scouts left the nest along a route so that the distribution of scouts was related to the distribution of finds of food in the past.

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STRIDULATION AND ASSOCIATED BEHAVIOUR IN CERTAIN ORTHOPTERA. 3. THE INFLUENCE OF THE GONADS

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Introduction

The previous papers in this series (Haskell, 1957, 1958) described the stridulation and associated behaviour of males and females of four related grasshopper species; this work raised many new problems, the most interesting of which are concerned with the sexual behaviour of the insects. For example, great interest attaches to the development of the "responsive state" in females (Haskell, 1958) in which the insect orientates to and moves towards a singing male, and the inhibition of this state by copulation and oviposition. Although the weight of evidence in insects (see Hanström, 1939) seems to be against any active controlling influence of the gonads, it was felt that these organs may play some role in the various behaviour patterns involved in courtship and copulation in the present grasshoppers and this paper describes experiments designed to investigate this speculation.

Materials and Methods

The species used were *Omocestus viridulus*, *Chorthippus brunneus* and *Chorthippus parallelus;* these were collected in the field at Imperial College Field Station, Berkshire, England, and reared under conditions described previously (Haskell, 1957, 1958). The apparatus for recording and analysing stridulation and for observing behaviour associated with it has also been described previously (Haskell, 1957). Each insect was individually marked, and was weighed daily; this enabled a general check on condition to be made, especially in relation to surgical operations and in the case of females allowed information on the oviposition cycle to be obtained. The definitions of stridulation and associated behaviour used by Haskell (1957) are retained in the present paper.

The Effect of the Gonads on Stridulation in Males

The experiments involved the extirpation of the gonads and associated glands in males; this was carried out, both in nymphs and adults, by making a small slit in the side of the abdomen about the level of the 6th segment and extracting the organs with fine, sharpened forceps, the wound being afterwards sealed with wax. Practice led to the perfection of this technique so that it was possible in most cases to remove not only the testes but the accessory glands with one movement of the forceps. Control for removal was made by killing the insects at the conclusion of the experiment and completely dissecting the abdomen to check for traces of sexual or accessory organs. Needless to say, mortality was high but sufficient numbers survived to enable a number of experiments to be carried out.

The castrated males were kept together in a breeding cage isolated from females; they were placed one by one in an observation cage (Haskell, 1957) and observed for spontaneous stridulation, response to playback of recorded stridulation, reponse to imitations of stridulation and response to and behaviour with females in the responsive state. Many insects, although not killed at once by the operation, died within a few days; it was found in general that those surviving for three days lived for the normal life-span although a proportion of nymphs surviving the operation subsequently died on moulting to adults. Because of the numbers involved and the time necessary for behavioural observations, only those insects which lived seven days or longer could be tested for responses with females.

As controls for this experiment, insects of the equivalent instar or adult age were collected in the field and a slit made in their abdomen of the same size and in the same position as in the castrates. Table I summarizes the results of this experiment; figures in this table do not include males which, on death and final dissection, were found to have portions of their testes and/or accessory glands still present in the abdomen. It will be seen that as far as spontaneous stridulation and behaviour with females was concerned, castration produced no very apparent effect. The song pattern and duration of both normal and courtship songs in castrated males was on the whole normal, regardless of whether the insects were castrated as adults or as nymphs; a few aberrations did occur but were of such a nature, as for example short duration of song, or small

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Table I. Effect of Removal of Gonads on Stridulation and Associated Behaviour in Various Stages of *C. parallelus* and *O. viridulus* Males.

	C. parallelus			O. viridulus
	3rd instar	4th instar	Adults	Adults
Number castrated	22	39	55	22
Number surviving	9	17	35	12
Number and % of survivors singing spontaneously	None	4 (28·4%) out of 14	23 (71·8%) out of 32	7 (58·3%) out of 12
Number and % of controls singing spontaneously under similar conditions as above	2 (20%) out of 10	3 (25%) out of 12	21 (84%) out of 25	7 (70%) out of 10
Number and % of survivors observed in courtship and/or copulation	—	2 (see text)	10 (52·6%) out of 19	4 (44·4%) out of 9
Number and % of controls observed in courtship and/or copulation under similar conditions as above	—	—	8 (66·6%) out of 12	3 (50%) out of 6

reduction of pulse repetition frequency, as to be explicable in terms of shock rather than any definite physiological change. The very fact that some of the castrated males sang normally and carried out courtship and copulation behaviour shows that the presence of the gonads does not exercise control of these behaviour patterns.

It was notable, however, that although on the whole courtship and copulation behaviour followed the patterns previously described, the activity of castrated males was reduced and the courtship behaviour was often enfeebled and much prolonged. This particularly applied to the duration of copulation; several pairs consisting of a normal female mated with a castrated male remained together for periods of from 2-10 hours, contrasting with the usual ½-2 hours of normal insects. This prolongation of copulation was almost certainly not due to the lack of any substance derived from the testes, since several implantations of living gonads were made into castrated males without altering the general behaviour. Rather is the cause of the prolongation to be looked for in the failure to produce and empty a spermatophore and this aspect is discussed later. In those castrated males which lived for their normal life span, there was no progressive enfeeblement, either in stridulation or in courtship behaviour. As far as nymphal behaviour is concerned, often in the fourth instar these stridulate and attempt to mate with females, and this was observed on two occasions with castrated fourth instar *C. parallelus*. Although there was no very marked species difference in response to castration, the males of *O.*

viridulus, despite their larger size, seemed more enfeebled than those of *C. parallelus*.

Effect of Gonads on Stridulation and Associated Behaviour in Females

Since the onset of the "responsive state" in female Acrididae is associated with maturation (Haskell, 1958; Renner, 1952) experiments relating to the influence of the gonads on this behaviour pattern were carried out. Two methods for assessing the influence of the ovaries and accessory glands were envisaged; the first was extirpation, the surgical and control techniques being the same as described above for males. The second method attempted was biochemical and depended on an observation made by Carlisle & Butler (1956) that ovarian growth in the honey-bee was stopped by injections of crustacean eye-stalk gland hormone. Ovarian inhibiting hormone from prawns was prepared in a variety of ways, in solution in ethyl oleate and as an emulsion in water using sodium lauryl sulphate as the dispersing agent and was injected into nymphal and adult females. Doses injected ranged from one to five eyestalk equivalents; since one eyestalk equivalent inhibits for one week the ovaries of a prawn of weight 2-5 gm., it was felt that the doses used should be effective, if active, in grasshoppers of weight of about 0·3 gm. However, no inhibitory action of any sort was detected; out of 15 fourth instar female *C. brunneus* and the same number of *C. parallelus* nymphs injected, 8 and 10 respectively moulted to adults and subsequently laid eggs. Out of 25 adult females of each species treated, 15 *C,*

brunneus and 12 *C. parallelus* subsequently laid viable egg-pods. Histological examination of ovarian tissue from experimental females showed no abnormalities.

Behaviour experiments had therefore to be confined to females whose ovaries had been extirpated; this was done, as with males, both in the third and fourth instar and with adults. However, mortality after extirpation in the nymphal stages was extremely high and since those surviving the operation lived only a few days, very few surviving the final moult, experiments with nymphs were abandoned. Mortality in adults was also fairly high but sufficient survived for several experiments.

Fourteen *C. parallelus* virgin adult females, already in the "responsive state", had their ovaries and accessory glands extirpated; they were tested with singing males every day for six days after the operation and the results are summarized in Table II. It will be seen that in three cases (Insects 3, 7, 11) the responsive state disappeared within 24 hours, in seven cases (Insects 1, 2, 4, 5, 8, 9, 14) it persisted for 48 hours and was then lost, and in the remaining four cases (Insects 6,10,12,13) it persisted for more than 48 hours. However, in Insect No. 6, the subsequent control dissection showed that the insect had retained a portion of ovariole tissue. Of the six control insects two (No. 16, 20) remained responsive for the rest of the experiment, three more (15, 17, 19) remained positive except when interrupted by oviposition and one (18) became unresponsive for unknown reasons for two days in the middle of the experiments.

In a further series of experiments with virgin adult *parellelus* females in the responsive state, the ventral nerve cord was cut between the 4th and 5th or 5th and 6th abdominal ganglia; out of eight insects which survived this operation for five days or more only two lost the responsive state, the remaining six actively responding to singing males.

The trend of these results was such as to suggest a non-nervous factor controlling the onset of the responsive state; the following experiment was therefore performed to find out if a factor was present in the blood affecting this behaviour pattern. Ten virgin adult *C. parallelus* females were castrated as before and their response behaviour checked daily; eight survived the operation for more than four days and as soon as they had lost the responsive state four were injected in the cervical membrane region with 2 cu. mm. of saline isotonic with their haemolymph.

Table II. Effect of Extirpation of Ovaries from Virgin Adult Females of *C. parallelus*.

Insect	Time in days					
	1	2	3	4	5	6
1	+ Op	+	—	—	—	—
2	+ Op	+	—	—	—	—
3	+ Op	—	—	—	—	—
4	+ Op	+	—	—	—	—
5	+ Op	+	—	—	—	—
6	+ Op	+	+	+	+	—
7	+ Op	—	—	—	—	—
8	+ Op	+	—	—	—	—
9	+ Op	+	—	—	—	—
10	+ Op	+	+	+	+	+
11	+ Op	—	—	—	—	—
12	+ Op	+	+	—	—	—
13	+ Op	+	+	+	—	—
14	+ Op	+	—	—	—	—
15	+ control	+	+	+	—	+ ov
16	+ control	+	+	+	+	+
17	+ control	+	— ov	—	+	+
18	+ control	+	—	—	+	+
19	+ control	+	+	+	— ov	—
20	+ control	+	+	+	+	+

Key: + responsive state; — loss of response state; Op when ovaries removed; Ov insect oviposited.

The remaining four were injected with 2 cu. mm. of haemolymph taken from a virgin adult *parallelus* female, with ovaries, which was in the responsive state. The results are summarized in Table III; it will be seen that although the surviving controls were uniformly negative in response, all the three surviving experimental animals showed a return to the responsive state for some period before death.

Inhibition of the Responsive State after Copulation

The responsive state in several grasshoppers is inhibited for periods of 2-24 hours by approaching oviposition and also by complete copulation

Table III. Injection Experiments with Castrated Adult Female *C. parallelus*.

Insect	Treatment	Time in days after operation				
		1	2	3	4	5
1	Injected 2 cu. mm. isotonic saline	—	—	—	—	Dead
2	,,	Dead				
3	,,	Dead				
4	,,	—	—	—	—	Dead
5	Injected 2 cu. mm. haemolymph of female in responsive state	—	+	+	—	Dead
6	,,	+	+	—	—	Dead
7	,,	—	+	+	—	Dead
8	,,	Dead				

Key: + responsive state; — responsive state lost.

(Haskell, 1958; Renner, 1952). Renner showed in *Euthystira brachyptera* that most probably the specific event causing inhibition after copulation was the filling of the receptaculum seminis of the female with sperm from the spermatophore. The question arises as to whether the cause of inhibition is a mechanical effect mediated by the nervous system, perhaps resulting from the mechanical stimulation of proprioceptors in the end-organ of the receptaculum consequent on its filling with seminal fluid, or a hormonal one, resulting either directly from the seminal fluid or as a result of its stimulation of the tissue of the receptaculum. This organ is innervated from the last abdominal ganglion and it is therefore reasonable to suppose that any nervous inhibition centering on it will be transmitted to higher centres by way of the ventral nerve cord. To test this supposition, seven *C. parallelus* females in the responsive state were allowed to copulate to completion with males; after 24 hours they were tested for responsiveness with singing males and were all negative. Their ventral nerve cord was then cut in the region of the 6th abdominal ganglion and the wound sealed with wax. Three died within 24 hours but four survived for three days during which time no response to singing males was observed. On dissection after death, the nerve

cord was found to be severed in all four insects and sperms were found in their receptacula.

An attempt was made to stimulate the receptaculum mechanically by injecting into it through a thin flexible glass tube and micrometer syringe 2 cu. mm. of isotonic saline; the injection was done successfully with four responsive *parallelus* females, but in no case was the responsive state inhibited. Attempts were also made to remove the receptaculum seminis but the operation was always followed by a moribund state so that copulation never took place in the experimental animals. Experiments in which mating pairs were parted at various times after the onset of copulation, the female then being tested for responsiveness and subsequently dissected, showed that responsiveness was never lost after copulation unless sperms were present in the receptaculum. In two cases it seemed that insertion of the neck of the spermatophore had begun and had been accompanied by the passage of some fluid, but no sperms were found in the receptaculum and the responsiveness of the females had not been terminated.

Discussion

The experiments on the influence of gonads on stridulation in the present paper are the more interesting in that the effects in males and females are so different. In males there is no effect; stridulation and copulation behaviour seemed to be unaffected; this was also noted by Regen (1909) in the case of castrated male *Gryllus campestris*. The observations of Husain & Baweja (1936) on the behaviour of desexualized adults of *Schistocerca gregaria* showed no alteration of courtship or mating behaviour, except that the duration of copulation was unduly prolonged. This was also noted during the present work; since copulation is normally terminated shortly after emptying of the spermatophore (Boldyrev, 1929), it seems likely that stimuli resulting from the transfer of sperm lead to the breaking-off of mating and the absence of a spermatophore occasioned by castration could induce abnormally long periods of copulation. However this may be, the important point is that in males the major elements of the stridulation/ courtship pattern were not altered by castration; in females the case is otherwise.

Consideration of the data of Table II, although rather sparse for this type of experiment, strongly suggests that castrated females in the responsive state lose this condition between 24-48 hours after the operation. The comparatively

slow onset of inhibition argues against this being mediated by nervous control and the related experiments on cutting the ventral nerve cord support this conclusion. The blood injection experiments (Table III) could clearly not stand by themselves but taken in conjunction with the previous two experiments support the hypothesis that some blood-borne chemical factor is responsible for onset of the responsive state in female *C. parallelus*. The work of Renner (1952) with castrated females of the grasshopper *Euthystira brachyptera* tends to the same conclusion; here also the insects underwent changes in their response pattern, the timing again suggesting hormonal control.

The inhibition of the responsive state by copulation has been demonstrated by Regen (1923) for crickets and by Renner (1952) and Haskell (1958) for grasshoppers; Renner's work suggested the filling of the receptaculum seminis with sperm as the causal stimulus and the present work supports this conclusion with the further implication that the inhibition is due to a chemical factor and is not directly mediated by the central nervous system. The two observations on responsive *C. parallelus* females which had been interrupted after partial copulation and in which fluid but no sperm was present in the receptaculum, suggests that it is in the seminal fluid itself that the controlling chemical factor will be found.

There is a widely held view (see Hanström, 1939, for many references) that the gonads of insects play little or no part in the development of secondary sex characteristics; morphologically speaking this is almost certainly true, as the frequent occurrence of gynandromorphs suggests and the present work does not suggest otherwise. However, much evidence is now available (see Engelmann, 1957) suggesting that the *function* of the reproductive system is controlled in many respects by hormones. For example, in Orthoptera the corpora allata control egg development and maturation and possibly an intragonadal hormone controls ovulation, and the work of Engelmann (1957) has demonstrated the presence of intra-organ hormone cycles involving the reproductive system.

It seems possible that some such system exists in some acridid grasshoppers and the present work, in agreement with that of Renner (1952), suggests that this is the case. The operation of this system affects sexual behaviour and since this must be basically controlled by higher nervous centres the ultimate point of action of the hormone or hormones involved must be on such centres, although perhaps indirectly. Speculative systems capable of explaining the present observed changes in sexual behaviour in grasshoppers could be erected but in view of the sparse information on copulation and fertilization mechanisms in Orthoptera this would be premature; the preliminary work herein described may, however, stimulate further work along the lines that the female gonads operate as one regulatory centre in a "hormone chain". The fact that crustacean eye-stalk hormone had no effect on grasshopper ovaries, although capable of inhibiting growth of the ovaries of honey-bees (Carlisle & Butler, 1956), is perhaps an indication that different orders of insects have evolved somewhat different hormone systems, at least biochemically.

Summary

1. Extirpation of testes and accessory glands in males of the acridid grasshoppers *C. parallelus* and *O. viridulus* did not alter their stridulation nor the behaviour associated with it; however, time spent in copulation was considerably increased and it is suggested that this may be due to the fact that in normal males the emptying of the spermatophore is the stimulus for the cessation of copulation.

2. Injections of crustacean eyestalk hormone, found to inhibit ovarian development in honey-bees, was without effect on ovarian tissue in the grasshoppers used.

3. Removal of ovaries and accessory glands in female *C. parallelus* in the "responsive state" resulted in the disappearance of this condition in from 24-48 hours. The responsive state was re-induced in several castrated females by injection of blood from females in the responsive state.

4. The responsive state was not terminated by cutting the ventral nerve cord in the abdominal region in normal females.

5. Inhibition of the responsive state in females by copulation only occurs after the passage of sperm into the receptaculum seminis; this inhibition is not mediated by the central nervous system, consequent on mechanical stimulation of the receptaculum seminis, and is probably due to some chemical factor associated with the sperm.

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HABITUATION OF THE POLYCHAETE *Nereis* TO SUDDEN STIMULI.
1. GENERAL PROPERTIES OF THE HABITUATION PROCESS

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Habituation to repeated stimulation is a widespread phenomenon and is regarded as one of the simplest forms of learning displayed by animals (Thorpe, 1956). Previous studies of habituation in polychaete worms have been devoted to the highly specialized tube-dwelling species, chiefly the sabellids and serpulids. These worms emerge partly from their tubes and spread a fan of branchial filaments which serve a respiratory and food-collecting function, and on stimulation they withdraw very rapidly. The worms have a very limited repertoire of responses and this represents their chief, and under natural conditions, almost their only one. A variety of sabellids and serpulids is reported to withdraw in response to such stimuli as a sudden decrease in light intensity, the passage of a shadow across them, to touch, or to mechanical shock (Nicol, 1950). Habituation to a sudden decrease in light intensity and to other stimuli has been reported in the sabellids *Bispira voluticornis* (Bohn, 1902), *Branchiomma vesiculosum* (Nicol, 1950), and the serpulids *Hydroides dianthus* (Hargitt, 1906; Yerkes, 1906), *Serpula vermicularis* (Hess, 1914) and *Mercierella enigmatica* (Rullier, 1948).

The "errant" polychaetes, though many of them live in burrows or tubes and may display a withdrawal reflex, are not as highly specialized for a tubicolous existence as are sabellids and serpulids, and they are more versatile in their behaviour. The Nereidae is the best known and most frequently studied family of "errant" worms. Most species of *Nereis* live in semi-permanent tubes and in several respects their behaviour is adapted to a tubicolous life (Clark, 1959). In particular, they display a withdrawal reflex on sudden stimulation. This sudden contraction of the longitudinal muscles of the body-wall may be observed in worms living in glass tubes, whether they have emerged from the mouth of the tube or are lying completely within it.

Previous investigations of nereids have established that they are photonegative (Bohn, 1902, Herter, 1926, Ameln, 1930) and swim out of the light into the shadow. In some species, e.g. *Nereis virens*, this photonegative response is abolished if the worms are provided with glass plates under which they may crawl (Loeb, 1913), in others, e.g. *N. diversicolor*, this is reported not to be so (Herter, 1926). In the present work it has been found that both *N. pelagica* and *N. diversicolor* will quickly adapt to continuous illumination and do not move into shaded areas of the tank if they are provided with glass tubes of suitable dimensions in which to live.

In this investigation of habituation in *Nereis*, the general properties of the process have been studied in *N. pelagica* and two classes of stimulus have been used: mechanical, in the form of mechanical vibration, and photic, in the form of increase and decrease in light intensity and moving shadows. Stimulation by light is the more amenable to control and standardization and the reactions of the worms to this type of stimulus have been studied in the greater detail.

Materials and Methods

Nereis pelagica (L.), used in these experiments, has been maintained in an aquarium under sea-water circulation and aeration. The worms are fed at irregular intervals on *Mytilus* and they appear to eat also any worms that die. They are provided with lengths of glass tubing of various sizes and the worms find their way into them and live in them, generally one worm to a tube. In the aquarium, the worms are in semi-darkness except when lights over the tanks are switched on for inspection or feeding. When required for experiments, a number of tubes containing worms is transferred to a shallow, white enamel pan of sea-water. Within 15-30 minutes of this disturbance the worms, whether illuminated or not, resume their normal activities. However, before any experiments have been carried out, the worms have been left for 1-2 hours under the experimental conditions to settle down. At the end of each experiment the worms have generaly been discarded, but occasionally were returned to the aquarium for use, chosen at random, in subsequent experiments. Only worms which have lived in glass tubes for some weeks have been used in experiments, so that having become accustomed to them, the worms' reaction

would be approximately normal.

The experiments were conducted in a north-facing room with the pan of worms screened from the window and illuminated by a light reflected from the walls. This dim light had an intensity varying between 0·08 and 0·10 ft-candles. Bright light was provided by a 40-watt white (Argenta) tungsten filament bulb in a white painted shade, suspended 18 in. above the worms. The light intensity from this source was 33·9 ft-candles. On occasions when less brilliant artificial light was required, the 40-watt bulb was replaced by a 15-watt sign lamp which gave an intensity of 4·7 ft-candles. The lights were connected directly to the public mains supply and may have varied slightly in their output. This has been neglected. Light intensities have been measured with an S.E.I. exposure photometer, using a standard white magnesium carbonate block.

Mechanical shock was produced by dropping a 6-oz. weight from a height of 18 in. onto the bench beside the worms.

In most experiments it has been found that not more than about 10 worms can be observed at the same time. Usually each experiment has been performed on one batch of 10 worms and then repeated on a second group of 10, and the results lumped together. Apart from the reaction to the initial stimulus in a series of trials, the average number of worms reacting in groups of five consecutive trials (expressed as a percentage of the total number of possible reactions) has been indicated in most of the figures. The percentage of worms reacting at each trial in the series has been plotted in Fig. 3.

Results

1. Habituation to Mechanical Shock

The response of *Nereis* to mechanical shock is nearly always a rapid contraction whether the worms are completely inside their tubes or not. Occasionally the worms respond with a slower contraction of the type more frequently seen as a response to a sudden increase in light intensity (see below). The stimulus was repeated at one minute intervals.

Habituation to mechanical shock is more variable than that to other stimuli. In some experiments (Fig. 1) only one-third of the animals react to the initial shock and thereafter habituation is very rapid. It is virtually total after the second stimulus and only occasional responses are shown up to the 12th or 13th stimulus, after

which habituation is complete. On other occasions (Figs. 8 and 9) more than half the worms react to the initial shock and a few reactions continue until the 30th or 35th stimulus.
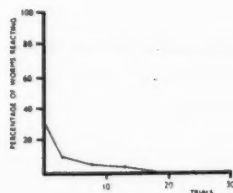
Fig. 1. Habituation to mechanical shock. Trials given at 1-minute intervals. Observations on 20 worms in 2 experiments. Points represent the average percentage reaction during 5 consecutive trials.

2. Habituation to a Sudden Decrease in Light Intensity

The worms were light-adapted for one hour before the beginning of the experiment. They were then habituated to a sudden decrease in light intensity from 33·9 to 0·08 ft-candles, produced by switching off the lamp. Trials were given at intervals of 1 minute and the light remained switched off for 9 seconds at each trial.

The reaction of the worms to a sudden decrease in light intensity is invariably a fast contraction. This is shown whether they are partly out of their tubes or completely withdrawn into them when the stimulus is applied. Two-thirds of the worms react to the initial stimulus, thereafter the number reacting falls almost to zero in the first 15 trials. Total habituation requires about 50 trials (Fig. 2, upper diagram).

3. Habituation to a Moving Shadow

Worms were light adapted as in the previous experiment and were then habituated to a shadow moving across them. The shadow was provided by sliding an opaque card across the lamp housing so that in about 0·5 second the light intensity fell from 33·9 to 0·08 ft-candles. The screen remained over the lamp for 9 seconds and tests were repeated at 1 minute intervals.

Initially the response to a moving shadow is a rapid contraction, but the worms habituate rapidly and, as they do so, the fast response is replaced by a slower contraction. After the 4th trial reactions to the shadow appear infrequently and these are almost invariably slow contractions. Habituation is complete by the 16th trial (Fig. 3).
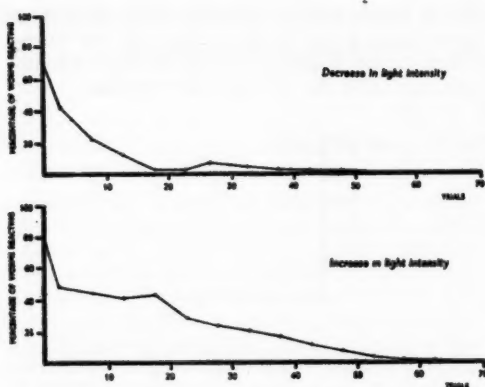
Fig. 2. *Upper diagram*. Habituation to a sudden decrease in light intensity from 33·9 to 0·08 ft-candles. Worms previously light-adapted. Trials given at 1-minute intervals, light switched off for 9 seconds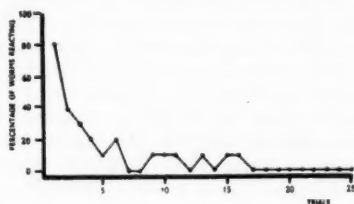 at each trial. Observations on 19 worms in 2 experiments. Points represent the average percentage reaction during 5 consecutive trials.
Lower diagram. Habituation to a sudden increase in light intensity from 0·08 to 33·9 ft-candles. Worms previously dark-adapted. Trials given at 1-minute intervals, light switched on for 9 seconds at each trial. Observations on 20 worms in 2 experiments.

Fig. 3. Habituation to a moving shadow causing a decrease in light intensity from 33·9 to 0·08 ft-candles. Worms previously light-adapted. Trials given at 1-minute intervals, light interrupted for 9 seconds at each trial. Observations on 10 worms. Points represent the percentage reaction at each trial.

4. Habituation to a Sudden Increase in Light Intensity

Worms were dark-adapted for 2 hours before the start of these experiments. They were then exposed to bright light (33·9 ft-candles) at intervals of 1 minute and the light remained switched on for 9 seconds at each trial. This represents a similar stimulus interval and duration to that given in the previous experiments.

The worms may react to a sudden increase in light intensity by a fast contraction, but more often they contract slowly and incompletely. The slow contraction begins after a slight delay and may not be complete until 2 seconds after the application of the stimulus. The entire longitudinal musculature may contract, or only the anterior or posterior part of the body be retracted. On occasion there may be a delay of 5 seconds or more between the application of the stimulus and the onset of contraction, but this appears to be a response to exposure to bright light rather than to a sudden increase in light intensity, as it does not appear if the light is switched on for only two or three seconds at each trial. In practice it is easy to distinguish between contractions which follow immediately after the application of the stimulus and those which are unusually delayed. Only the former have been included in these results, though as the delayed response disappears before the worms are habituated to the stimulus, its inclusion would not affect the results.

Four-fifths of the worms react to the first stimulus, thereafter the number reacting falls off gradually, but habituation is not complete until after more than 60 trials (Fig. 2, lower diagram). There are very few responses after the 50th trial. These results should be compared with those of the converse experiment in which the worms were habituated to a sudden decrease in light intensity (Fig. 2, upper diagram). The worms habituate much faster to a decrease than to an increase in intensity.

5. Influence of Exposure to Light on the Habituation Rate

In the previous set of experiments anomalous results were obtained at first, when the length of time the light was switched on at each trial was not standardized. These anomalies suggested that the reactions of the worms were determined not only by the sudden increase in light intensity, but also by continued exposure to the light, and this view was strengthened by the fact that if the light remained switched on for more than 2-3 seconds, a delayed contraction sometimes appeared. The worms were therefore habituated to sudden increases in light intensity as before, with trials at 1-minute intervals, but the length of time the light remained switched on was changed in successive experiments. The total exposure of the worms to light ranged between 3 and 48 seconds per trial (5-80 per cent. of the total duration of the experiment) and the relation between habituation rate and exposure to light is shown in Fig. 4. Habituation is rapid when the expos-

ure to light is low, but the number of trials increases rapidly to a maximum at 30 per cent. exposure, when 88 trials are necessary to produce complete habituation. As the exposure to light at each trial increases from 30 per cent. to 80 per cent. there is a steady decrease in the number of trials necessary for habituation.
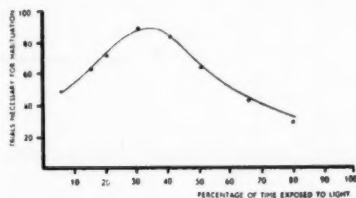

Fig. 4. Influence of increased exposure to light on the habituation rate. Worms habituated to a sudden increase in light intensity from 0·08 to 33·9 ft-candles. Trials given at 1-minute intervals.

Two factors seem to be responsible for this phenomenon, one tending to increase the habituation rate, the other to decrease it. On exposure to bright light the worms become light-adapted, and the adaptation becomes more complete the greater proportion of the time the worms are exposed to light. Presumably a state of light-adaptation diminishes the effectiveness of the stimulus and so increases the rate of habituation to it. On the other hand, light of this intensity appears to represent a very strong stimulus. It has been found impossible to establish a conditioned response in worms when light of this intensity is used as the conditioning stimulus (Clark & Grant, unpublished observations). Up to the point where adaptation becomes an effective factor, increased exposure to this strong stimulus makes habituation more difficult.

6. Influence of the Frequency of Trials on the Habituation Rate

Worms were habituated to a sudden increase in light intensity after being dark-adapted for 2 hours. Trials were given at intervals of $\frac{1}{2}$, 1, 2, 3 and 5 minutes, respectively, in successive experiments, and in each case the exposure to light represented 5 per cent. of the total time. The number of trials necessary to produce complete habituation increases with the interval between tests (Fig. 5).

7. Persistence of Habituation

Worms were dark-adapted and then exposed
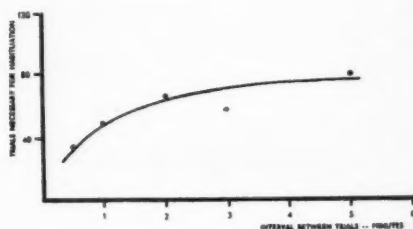
Fig. 5. Influence of the interval between trials on the habituation rate. Worms habituated to a sudden increase in light intensity from 0·08 to 33·9 ft-candles. Light switched on for 5 per cent. of the interval between trials. Observations on 30 worms.

to bright light for 12 seconds at 3-minute intervals. They were completely habituated and then left in dim light for 1 hour, when they were habituated again in the same manner. After a further period of 17 hours in dim light they were habituated a third time, and after a further $5\frac{1}{2}$ hours, a fourth. Fig. 6 shows the habituation curves obtained 1, $5\frac{1}{2}$ and 17 hours after previous habituation. Between 60 and 80 per cent. of the worms react to the initial stimulus in each series of tests, but 1 hour after previous habituation the worms can be almost completely habituated again after only 12 trails. After $5\frac{1}{2}$ hours, 23 trials are necessary to produce substantial habituation, and after 17 hours, the habituation curve is restored almost to its original form.

These experiments were repeated with the difference that the interval between trials was reduced to half-a-minute and the length of time the worms were illuminated was proportionally reduced to 2 seconds at each trial. In this case habituation persists for less than $5\frac{1}{2}$ hours (Fig. 7).

No systematic studies have been made of the persistence of habituation to stimuli other than a sudden increase in light intensity, but some pertinent information is included in the following section. Habituation to a moving shadow, the stimulus being applied at 1-minute intervals, totally disappears within 40 minutes (Fig. 9, lower diagram). Habituation to a sudden decrease in light intensity, with the same frequency of stimulation, is still substantially complete 45 minutes after the last trial of the series (Fig. 11, lower diagram).

8. Independence of, and Interaction Between Habituation to Different Stimuli
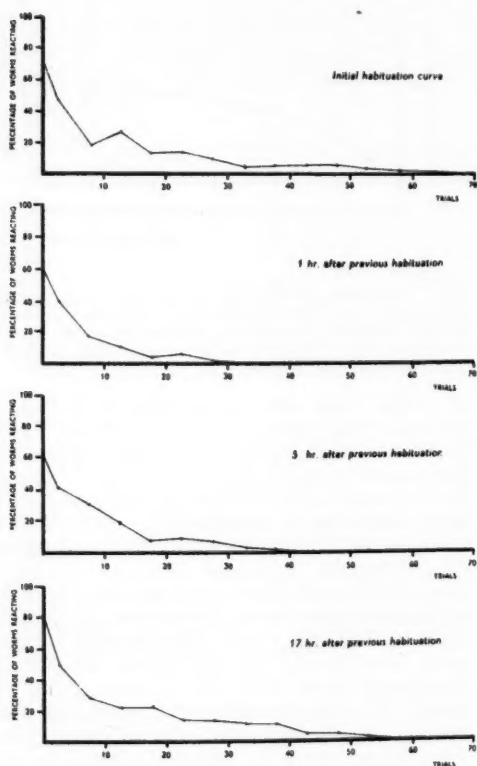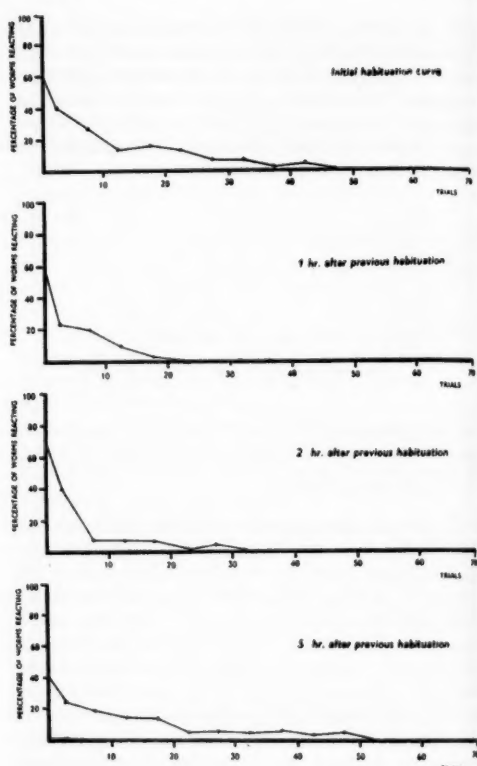
Habituation to mechanical shock, a sudden

Fig. 6. Decay of habituation to a sudden increase in light intensity from 0·08 to 33·9 ft-candles. Worms dark-adapted before the first experiment and left in dim light between succeeding experiments. Trials given at 3-minute intervals, light switched on for 12 seconds at each trial. Observations on 10 worms.

Fig. 7. Decay in habituation to a sudden increase in light intensity from 0·08 to 33·9 ft-candles. Worms dark-adapted before the first experiment and left in dim light between succeeding experiments. Trials given at ½-minute intervals, light switched on for 2 seconds at each trial. Observations on 9 worms.

increase or decrease in light intensity, or to a moving shadow occurs at a different rate in each case, suggesting that each is an independent process. This can be directly established for some stimuli. Worms habituated to a moving shadow are not thereby habituated to a mechanical shock, and *vice versa*. The habituation rate to either stimulus is the same whether the worms have previously been habituated to the other or not (Figs. 8 and 9).

It is perhaps not surprising that habituation to such dissimilar stimuli as mechanical shock and a moving shadow should be independent processes, but when habituation to two very similar stimuli are compared, some degree of interference between the two processes appears. A

decrease in light intensity produced by suddenly switching off the light, or, more slowly, by sliding a screen across the lamp, are two similar stimuli to which the worms habituate at different rates.

The stimuli differ from each other in two respects: rate of change of light intensity, and movement. Although the habituation rates to the two stimuli are different, the two habituation processes interact with each other as the following experiments show.

Worms were light-adapted for 2 hours and then stimulated by decrease in light intensity at 1-minute intervals; the light remained dim for 9 seconds at each trial. For the first 5 trials decrease in intensity was produced by moving a screen across the lamp, for the next 105 trials
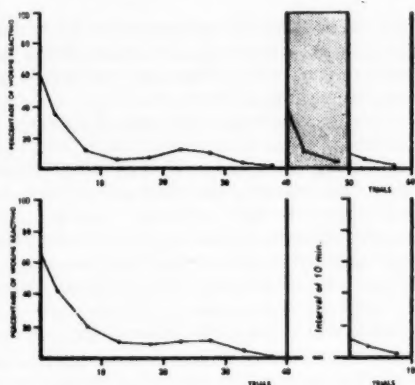
Fig. 8. Independence of habituation to mechanical shock (unstippled) and to a moving shadow (stippled). Worms light-adapted and illuminated throughout the experiment. Trials given at 1-minute intervals. Observations on 20 worms in 2 experiments.

Upper diagram. Uninterrupted stimulation, first by mechanical shock and then by moving shadow causing a decrease in light intensity from 33·9 to 0·08 ft-candles. Light obscured for 9 seconds at each trial.

Lower diagram. Two periods of stimulation by mechanical shock, separated by an interval of 10 minutes without stimulation.

Fig. 9. Independence of habituation on a moving shadow and to mechanical shock. See Fig. 8 for details.

into three phases. Between the 6th and 10th trials the percentage of worms reacting to the new stimulus falls still further, but this phase of depressed responsiveness ends abruptly (at A in Fig. 10), and by the 15th trial, an average of 22 per cent. of the worms react. This period of enhanced responsiveness, during which between 20 and 30 per cent. of the worms react at each trial, continues for a further 30 trials (ending at B,

the decrease was produced by switching off the lamp, and finally for the last 10 trials by moving a screen across the lamp again (Fig. 10, upper diagram).

During the first 5 trials, habituation follows its usual course and the worms are almost completely habituated to a moving shadow in that time. The response of the worms to a sudden decrease in light intensity, beginning at the 6th trial, is long and complicated. It can be divided

Fig. 10. Interactions between habituation to a moving shadow (stippled) and to a sudden decrease in light intensity (unstippled). Worms previously light-adapted. Trials given at 1-minute intervals, light switched off or obscured for 9 seconds at each trial. Decrease in light intensity from 33·9 to 0·08 ft-candles. Observations on 20 worms in 2 experiments.

Upper diagram. Uninterrupted stimulation. A is the end of the period of depressed response, B the end of the period of enhanced response and the start of habituation.

Lower diagram. Stimulation by a moving shadow for two periods of 30 trials, separated by an interval of 105 minutes in bright light without stimulation.

Fig. 10). At the 45th trial from the start of the experiment, the number of worms reacting begins slowly to decline, but complete habituation requires an additional 60-65 trials. After this the worms are found still to be habituated to a moving shadow, though habituation to this stimulus does not persist for 105 minutes under conditions of constant illumination (Fig. 10, lower diagram). Its persistence in the former experiment must be attributed to repeated stimulation by sudden decrease in light intensity. The entire experiment has been repeated several times with different groups of worms, giving identical results. The time course of events is the same in each case and Fig. 10 has been constructed from the combined results of two such experiments.

The converse experiment has also been performed (Fig. 11) in which light-adapted worms were habituated first to a sudden decrease in light intensity and then exposed to a moving shadow. The worms are completely habituated to a sudden decrease in intensity by the 50th trial. On stimulation by a moving shadow at the 61st trial, 20 per cent. of them react. A small number of worms continue to react to subsequent stimuli and habituation is not complete until the 105th trial from the start of the experiment. On stimulation by a sudden decrease in light intensity after the 105th trial, a three phase response, in miniature, appears, and habituation is not complete until the 145th trial. If the worms are exposed to a sudden decrease in light intensity for 60 trials and then left under con-

ditions of constant illumination for 45 minutes (Fig. 11, lower diagram), it is found that a substantial degree of habituation persists in the absence of periodic stimulation.

The results of the second series of experiments are different from those of the first. The intervening period of exposure to a moving shadow, far from maintaining the habituation to a sudden decrease in light intensity, increases the sensitivity of the animals to this stimulus. During the second period of exposure to a sudden decrease in light intensity, there is even a three-phase sequence of events on a miniature scale, with an initial depression of response, followed by an increase in response before habituation begins. Habituation to a moving shadow when following habituation to a sudden decrease in light intensity (Fig. 11), does not show a three-phase sequence, but it does take nearly twice as long as usual.

Considering these experiments as a whole, we may conclude: 1. Habituation to a sudden decrease in light intensity and to a moving shadow are separate but interacting processes.

2. Exposure to a sudden decrease in light intensity at regular intervals maintains an already established state of habituation to a moving shadow, but stimulation by a moving shadow does not maintain habituation to a sudden decrease in light intensity.

3. If the worms are already habituated to either stimulus, they are more resistant to habituation to the other.
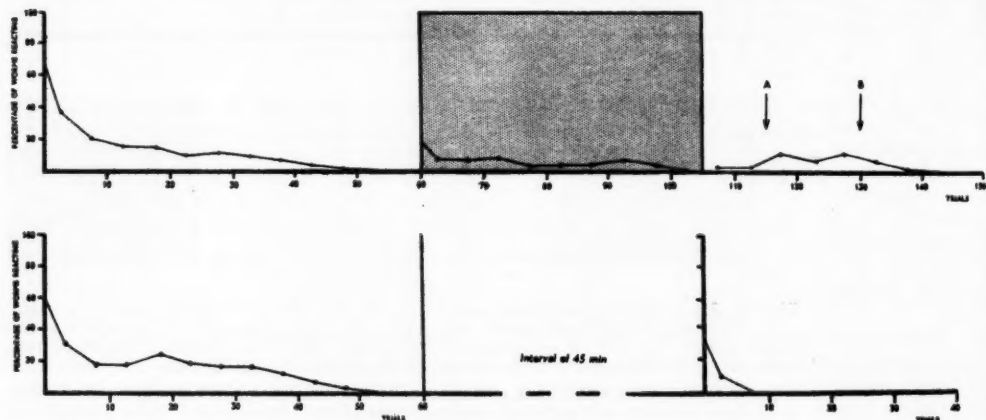
4. If the worms are habituated to a moving

Fig. 11. Interactions between habituation to a sudden decrease in light intensity and to a moving shadow. See Fig. 10 for details.

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shadow and are then exposed to a series of trials in which the light intensity is suddenly decreased, a three-phase response is seen: first, a period of depressed response, then one of enhanced response, and finally, slow habituation.

9. Latent Habituation

Another type of interaction between habituation processes occurs in response to stimuli of the same kind but of different intensity. The stimulus used in these experiments was a sudden increase in light intensity, and the animals were dark-adapted for 2 hours before tests were carried out. Worms which had not previously been habituated in any experiment, were exposed to a sudden increase in light intensity from 0·08 to 4·7 ft-candles at 1-minute intervals. The light remained switched on for 12 seconds at each trial. The worms were subjected to 60 tests with this stimulus and then, immediately afterwards, were stimulated for 60 trials by a sudden increase in light intensity from 0·08 to 33·9 ft-candles.

The worms do not react to the slight increase in light intensity during the first 60 trials. They do react to the stronger stimulus during the second half of the experiment, but they habituate rapidly to it (Fig. 12, lower diagram). In less than 20 trials habituation is almost complete and no reaction occurs after the 41st trial. This is in contrast to the normal course of habituation to the stronger stimulus. When tested two days after the previous experiments, the same worms required 70 trials before becoming habituated to a sudden increase in light intensity from 0·08 to 33·9 ft-candles (Fig. 12, upper diagram). Thus, repeated stimulation of too low intensity to evoke a reaction brings about a state of partial habituation to a stronger stimulus of the same kind.

Discussion

The failure of the worms to respond after a number of trials with a given stimulus is not due to neuromuscular or sensory fatigue, but must be a central process (i.e. habituation). Neuromuscular fatigue is precluded by the fact that when worms have been habituated to one stimulus, they can be made to react again in the same manner as before if the nature of the stimulus is changed. Furthermore, exposure to a weak stimulus, which elicits no visible response, produces a partial inhibition to a stronger stimulus of the same kind. Sensory fatigue is unlikely to be the cause of the decline in responsive-
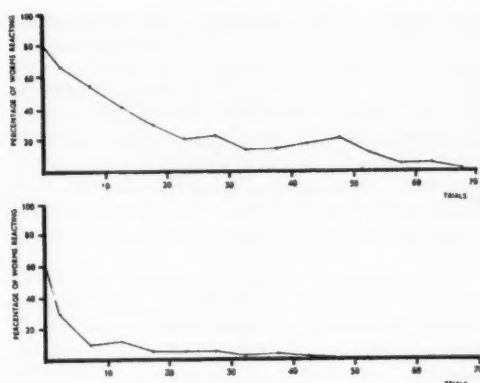

Fig. 12. *Upper diagram.* Normal course of habituation to stimulation by a sudden increase in light intensity from 0·08 to 33·9 ft-candles. Trials given at 1-minute intervals, light switched on for 12 seconds at each trial.

Lower diagram. Habituation of the same worms to a sudden increase in light intensity from 0·08 to 33·9 ft-candles immediately after exposure to 60 stimulations by sudden increase of light intensity from 0·08 to 4·7 ft-candles. Trials given at 1-minute intervals, light switched on for 12 seconds at each trial. Observations on 12 worms.

ness because a slight change in the nature of the stimulus may cause a reappearance of the response although the same sense organs are involved. Both neuromuscular and sensory fatigue are unlikely because of the relatively long duration of the state of reduced responsiveness.

Habituation to sudden stimuli occurs in a great many, if not all, animals, and from the study of this phenomenon a number of general characteristics of the habituation process have been recognized: it is a function of the elicitation rate, cumulative, impermanent and may, in some cases, be generalized (Hilgard & Marquis, 1940). It has been taken to be an active process rather than a mere dropping out of responses (Humphrey, 1933) and in several respects it is akin to the extinction of a conditioned response (Hilgard & Marquis, 1940). These characteristics are not necessary features of habituation, which may be defined simply as "the . . . waning of a response as a result of repeated stimulation in the absence of reinforcement" (Thorpe, 1956). There is no reason to suppose that habituation should necessarily involve the same processes, of which the general characteristics enumerated above are presumably a reflection, in all animals. It is difficult, for example, to understand in what sense habituation could be regarded as a "cen-

tral process" in organisms like protozoans which do not possess a central nervous system. Most of the previous studies of habituation, and those from which the general characteristics of the phenomenon have been derived, have been on vertebrates. The polychaete worms have a central nervous system of a much lower grade of structural organization than any vertebrate, and it is of some interest to find that the same general characteristics of the habituation process occur in *Nereis* as in higher animals.

Habituation in *Nereis* is achieved with fewer trials if the trials are more closely spaced (Fig. 5) and the greater the number of trials necessary to produce habituation, the longer the habituation persists (Figs. 6 and 7), though it persists for only a limited period. Thus, habituation is impermanent as in other animals. Hilden (1937) and Brogden, Lipman & Culler (1938) showed that the extinction process continues and can be detected by suitably sensitive instruments, long after gross reaction ceases. This is perhaps the explanation of the partial habituation to a strong stimulus as a result of previous exposure to repeated exposure to a weaker stimulus of the same kind in *Nereis* (Fig. 12), although the worm does not react visibly to the weaker stimulus. The habituation process may sometimes be generalized, that is to say, habituation to one stimulus in a decreased response to another (Coombs, 1938; Porter, 1938). Such a phenomenon has not been found in *Nereis*, but insofar as generalization of habituation implies an interaction between two distinct habituation processes, something of the same kind has been detected. There is no interaction between the habituation to widely differing stimuli, such as a moving shadow and mechanical shock, but there is between two similar stimuli, such as a moving shadow and a sudden decrease in light intensity (Figs. 10 and 11). The interactions effects are complex and not reciprocal, and it is not clear what interpretation should be placed on these results, except that habituation to one stimulus makes habituation to the other more difficult.

Summary

1. The polychaete worm *Nereis pelagica* habituates most rapidly to repeated stimulation by a moving shadow, or by mechanical shock. It habituates less rapidly to a sudden decrease in light intensity and most slowly to a sudden increase in light intensity.

2. The rate at which worms habituate to a sudden increase in light intensity is dependent upon the length of time the light remains switched on at each trial. It habituates most slowly when the light remains on for 30 per cent. of the time at each trial.

3. Habituation becomes slower as the interval between trials increases.

4. Habituation to a sudden increase in light intensity, with trials given at 3-minute intervals, persists for about 17 hours. If trials are given at $\frac{1}{2}$-minute intervals, habituation persists for about 5 hours.

5. Habituation to a moving shadow is independent of habituation to mechanical shock, but habituation to a moving shadow and to a sudden decrease in light intensity are mutually interacting processes. Worms already habituated to one require a greater number of trials than usual before they become habituated to the other. Exposure to stimulation by a sudden decrease in light intensity maintains an already existing state of habituation to a moving shadow, though the reverse is not true.

7. If the worms are repeatedly exposed to a stimulus too weak to evoke a visible response, they become partially habituated to a supraliminal stimulus of the same kind.

8. Habituation is a function of the elicitation rate, is cumulative and impermanent in *Nereis* as in other animals. Generalization of habituation has not been observed.

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HABITUATION OF THE POLYCHAETE *Nereis* TO SUDDEN STIMULI.
2. BIOLOGICAL SIGNIFICANCE OF HABITUATION

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Introduction

In the first part of this paper (Clark, 1960), the general properties of habituation of the withdrawal reflex of *Nereis* were examined. In this second part, the biological consequences of habituation in these worms will be considered.

The withdrawal reflex is simple and stereotyped: the longitudinal muscles of the body wall contract abruptly while the circular muscles relax. The body is shortened and, so long as part of it is anchored, the exposed parts of the worm are withdrawn into the shelter of the tube in which it lives. The only variations that occur are in the rate and extent of contraction. Any sudden stimulus will evoke it, even in the Nereidae which are among the more versatile polychaetes, and the reflex can be evoked as readily when the worms are completely inside, or completely outside their tubes, as when they are partly within them. In the former cases, of course, the contraction serves no useful purpose. A similar reflex occurs among a great many other annelid worms and in most of the species that have previously been studied, it is much more highly developed than in *Nereis*.

The withdrawal reflex of tubicolous polychaetes, particularly in response to moving shadows or to a sudden decrease in light intensity, has generally been interpreted as an anticipatory response to the approach of a predator (Jenkins, 1940). The withdrawal of the worm interrupts a number of maintenance activities, including feeding, tube-building and, sometimes, respiration. It is important therefore that unnecessary withdrawals elicited by accidental stimuli should be reduced to a minimum. Habituation to repeated but unreinforced warning stimuli may be regarded as a mechanism by which this end is achieved, and Nicol (1950) expressed a widely held view when he interpreted the slow habituation of the sabellid worm, *Branchiomma vesiculosum*, to moving shadows as "a compromise between the conflicting needs of the organism, withdrawal from its enemies, and maintained expansion for feeding and respiration."

Three features of habituation that appear to be of biological significance have emerged from previous studies of the withdrawal reflex in polychaetes. They are:

1. the habituation rate is related to the severity or the significance of the stimulus;
2. the habituation rate, or conversely the re-activity of the worms, is related to the normal background of environmental stimulation that the animals experience;
3. the animals are particularly sensitive to changes in the prevailing pattern of background stimulation.

A consideration of these and other factors may help to explain how the "compromise between the conflicting needs of the organism" is reached. For, unlike some animals that react indiscriminately to sudden stimuli until they learn by experience which are innocuous and then no longer react to them, a tubicolous polychaete must distinguish between stimuli which in one context are innocuous and in another are potentially dangerous (Nicol, 1950). A moving shadow, for example, may be caused by an approaching predator or by sea-weed floating on the water.

Thus there are two opposing aspects of modification of the withdrawal reflex to consider. One is concerned with conditions under which the reflex disappears (habituation), the other with conditions under which it is manifested (loss of habituation or failure to habituate).

An examination of the withdrawal reflex of *Nereis* from this point of view forms the present paper.

Behaviour of Individual Worms

In the interpretation of habituation curves, based on the average performance of populations of animals, much depends upon the behaviour of individuals. As an example, different interpretations would be placed on a slow rate of habituation of a population of worms if it was found to be due to the continued reactions of a single animal or to the sporadic and less frequent reactions of all the members of the population.

Fig. 1 shows the reactions of 15 *Nereis pelagica*

to repeated stimulation by a sudden decrease in light intensity. The habituation curve for the whole population is shown on the right, the reactions of individuals on the left. There is very great variation in the reactivity of the worms.
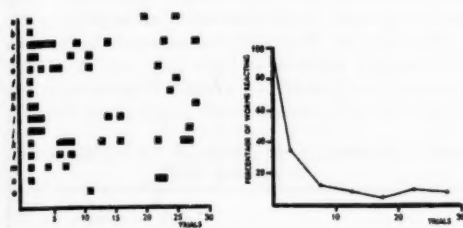

Fig. 1. Habituation of *N. pelagica* to a sudden decrease in light intensity from 33·9 to 0·1 ft-candles. *Left:* individual responses: *right:* habituation curve. Points represent the average percentage reaction during 5 consecutive trials. Trials given at 1-minute intervals.

Two specimens (*c* and *k*) reacted to 9 of the 30 stimuli, and two (*b* and *o*) reacted only once. The pattern of reactions during the 30 trials also differs markedly from one specimen to the next. Some (e.g. *c* and *e*) reacted to several of the first few stimuli and then at a decreasing rate to subsequent stimuli. One (*k*) reacted at approximately the same rate throughout the series, while others (*l* and *m*) reacted to a few stimuli and then ceased to react at all.

Not only does one worm show a different pattern of reaction from the others, but the course of habituation of a single individual varies on different occasions. Fig. 2 shows the reaction of 7 worms during habituation to a sudden decrease in light intensity on two separate occasions. One worm (*a*) showed a similar pattern of reactions on both occasions, but others (e.g. *c*, *d*, and *e*) which reacted a great deal dur-
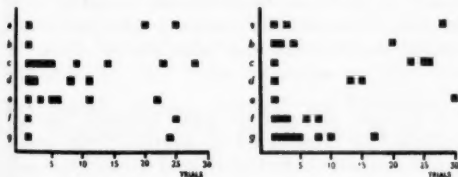
Fig. 2. Habituation of *N. pelagica* to a sudden decrease in light intensity. *Left:* individual responses during initial habituation; *right:* individual responses of same worms on the following day. Trials given at 1-minute intervals.

ing the first series of trials, reacted only occasionally during the second series. Some (e.g. *f* and *g*) reacted very little during the first series, but frequently during the second.

Most worms contract more or less violently, particularly if they are engaged in some form of activity (pumping, crawling along the tube, partly extended from it and searching around its mouth, etc.) when first stimulated. The worms that do not react are generally those which are quiescent at that time. The length of time that passes before the worms extend again and resume their normal activities varies widely from individual to individual. More than half the worms recover within 1 minute, but the remainder may remain contracted for up to 19 minutes (Fig. 3). The length of time the worms remain contracted bears little relation to the nature of the stimulus, and the same proportion of slow recoveries is observed whether the stimulus be a sudden decrease in light intensity, a moving shadow, mechanical shock, or a combination of them.
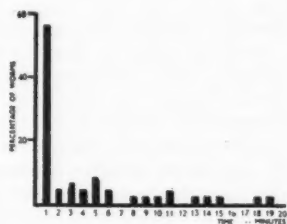

Fig. 3. Time taken for recovery from contraction after the initial stimulus of a series of trials. Trials given at 1-minute intervals, various stimuli employed.

Of the worms that are active, or at least extended, during trials subsequent to the first, very few continue to react with any regularity. A single specimen in one experiment reacted to each of the first 12 trials, but this is exceptional (Fig. 4). Once the worms have recovered from the initial stimulus in a series, they react only sporadically and very rarely to 2 or more consecutive stimuli. Recovery from each of these later contractions is very rapid.

Rate of Habituation to Different Stimuli

From previous studies of habituation in polychaetes, chiefly serpulids and sabellids, there appears to be some relationship between the rate of habituation and the nature of the stimulus. Nicol (1950) found that *Branchiomma* habituates
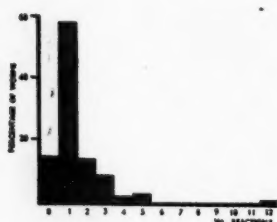
Fig. 4. Number of reactions from the start of a series of trials before the worms first fail to respond. Trials given at 1-minute intervals, various stimuli employed.

rapidly to a sudden decrease in light intensity, produced by switching off a light, more slowly to a moving shadow, and not at all to tactile stimulation. Comparable behaviour has been reported in the serpulid *Mercierella enigmatica*, although it habituates more rapidly to all stimuli than *Branchiomma*. It habituates readily to a moving shadow, more slowly to mechanical shock produced by tapping the side of the vessel in which the worms were kept, and most slowly of all to the simultaneous presentation of mechanical shock and a moving shadow (Rullier, 1948). *Hydroides dianthus* also habituates more slowly to tactile stimulation than to a moving shadow (Yerkes, 1906). All these stimuli represent potentially dangerous situations and may presage the approach of a predator. Nicol (1950) pointed out that a moving shadow is biologically a more significant stimulus than an equivalent, though sudden, decrease in light intensity because predation necessarily involves movement and this quality is missing from the latter stimulus. It appears that the more biologically meaningful the stimulus, the more slowly the worms habituate to it, and it can obviously be argued that this is likely to have a distinct survival value for the worms.

Tactile stimulation, to which the sabellids and serpulids are so sensitive, evokes only a very slight withdrawal in *Nereis pelagica*. If the prostomium or tentacular cirri of a worm that has partly emerged from its tube are tapped with a thin wooden splint or a glass rod, the worm withdraws its anterior end into the tube, but the contraction is confined to the longitudinal muscles of a few anterior segments and is not to be compared with the startle reflex shown in response to other stimuli. Naturally, if the worm is tapped hard enough, a more violent response can be elicited. The worm quickly emerges again and may withdraw on three or four more occasions, but after that it attempts to grasp the object

with its jaws and may even be lured further out of its tube by teasing it with the rod. This localized disturbance will cause worms in neighbouring tubes to rush to the end of them and to emerge. These reactions are more marked if the worms are hungry than if they have recently been fed, and are connected with feeding behaviour. They can be ignored in the following discussion.

The rate at which *Nereis pelagica* habituates to a sudden increase in light intensity, a sudden decrease in intensity, to a shadow moving across it, and to mechanical shock is given in Table I.

Table I. Influence of the Nature of the Stimulus on the Habituation Rate.

Stimulus	Trials necessary for habituation*	
	Substantial habituation	Total habituation
Mechanical shock combined with sudden decrease in light intensity	45+	65+
Sudden increase in light intensity	40	60+
Sudden decrease in light intensity	15	50
Moving shadow	4—5	15—20
Mechanical shock†	2—3 6—10	12—13 30—35

*Trials given at 1-minute intervals in each case.
†Habituation to mechanical shock shows greater variation than habituation to other stimuli. Fastest and slowest rates of habituation are given in the table.

So far as these stimuli may be regarded as tokens of the approach of a predator, it appears at first sight that *Nereis*, unlike serpulids and sabellids, habituates most slowly to the least urgent stimuli and most rapidly to the most urgent warning stimulus. On closer examination, it can be seen that these habituation rates have some biological significance and that the behaviour of *Nereis* is essentially comparable with that of sabellids and serpulids.

A sudden increase of light intensity should probably be considered separately from other stimuli, since it cannot be related to the approach of a predator whereas the others can. Indeed, *Hydroides dianthus* (Hargitt, 1906; Yerkes, 1906), *Serpula vermicularis* (Hess, 1914) and *Branchiomma vesiculosum* (Nicol, 1950), all of which have well developed withdrawal reflexes,

do not react at all to a sudden increase of light intensity. Such a stimulus is usually experienced in nature only when the worms are suddenly illuminated by sunlight. Many unprotected animals avoid exposure to the harmful effects of ultra-violet radiation by their photonegative behaviour. Sabellid and serpulid worms do not emerge from their tubes unless they are submerged, so that they are effectively screened by a layer of water. *Nereis*, on the other hand, is an intertidal animal and may emerge from its tube when it is exposed or covered by only very shallow water. Its persistent response to a sudden increase in light intensity may therefore be adaptive and protect it from exposure to solar irradiation.

Although a moving shadow is a more complex stimulus than a sudden decrease in light intensity, the latter is the stronger stimulus in that the rate of change of light intensity is greater. *Nereis* appears to be sensitive to the rate of change of light intensity, for if the screen is moved across the lamp slowly enough, e.g. over a period of 2-3 seconds, the worms never react to it. When the shadow passes across them rapidly they react initially to it, but habituate to the stimulus after a few repetitions. A considerably greater period is required for habituation to a sudden decrease in intensity produced by switching off the light.

A stimulus of greater urgency can be provided by combining a sudden reduction in light intensity with mechanical shock. In trials in which both stimuli were presented simultaneously at 1-minute intervals, 10 per cent. of the worms continued to react even after 65 trials. The rate of habituation to either stimulus alone is considerably greater (Fig. 5).

Thus in *Nereis*, as in the sabellids and serpulids, there is a hierarchy of stimuli of increasing intensity or urgency, to which the animals habituate less and less readily. The stimuli in question are different in *Nereis* from those in the other polychaetes, but in all of them there is a suggestion that the more immediate the warning stimulus, the less readily the worms habituate to it.

Relationship Between Habitat and the Rates of Habituation to Repeated Stimulation

The reactions of worms to sudden stimulation depends, in part, upon the type of stimuli to which they are exposed in their normal environment. Hargitt (1909) found that specimens of *Hydroides dianthus* obtained from deep water where the light is perpetually dim, do not react
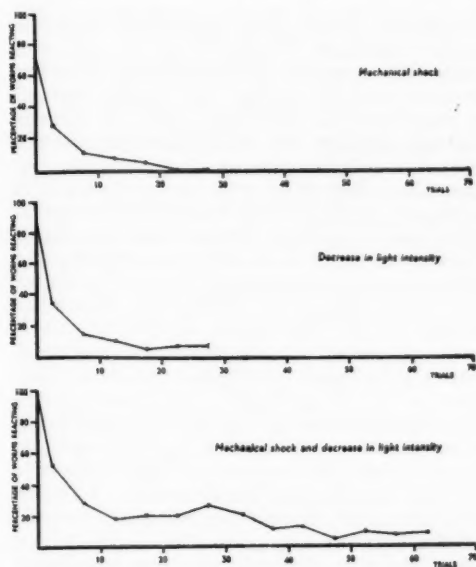

Fig. 5. Habituation of *N. pelagica* to mechanical shock, to a sudden decrease in light intensity, and to a combination of both stimuli presented simultaneously. Trials given at 1-minute intervals. *Top:* observations on 26 worms; *middle:* on 28 worms; *bottom:* on 16 worms.

to moving shadows, unlike specimens of this species from shallow water. The sabellids *Sabella pavonina* and *Spirographis spallanzanii* are nocturnal (Fox, 1938) and the former, at least, has poorly developed shadow reflexes compared with *Branchiomma vesiculosum* which expands and feeds during the day (Nicol, 1950). In some species, comparable changes in the reactions of individuals may be induced by suitable treatment of the animals. Specimens of *Hydroides dianthus*, taken from shallow water and kept in dim light over the winter, have a reduced sensitivity to moving shadows (Hargitt, 1909) and prolonged exposure of *Hermella* (=*Sabellaria*) *ascolata* to bright sunlight reduces its sensitivity also to shadows (Bohn, 1902).

Nereis pelagica lives in the intertidal and shallow sublittoral zone of rocky shores. The beaches it inhabits have a fairly dense growth of sea-weeds and often the worms live beneath the algal holdfasts. In such an environment the worms are exposed almost continually to moving shadows produced by the weed and to mechanical shocks caused by waves breaking, for rocks and sea-weeds are found only where there is a moderate degree of wave action. *Nereis*

diversicolor, on the other hand, is an estuarine species which lives in galleries in intertidal mud banks. Here there are neither breaking waves nor sea-weeds and the animal is not usually exposed to shadows or to mechanical shocks in its normal environment. The structural differences between *N. pelagica* and *N. diversicolor* are insignificant, and, apart from their differences in ecological preferences, no material differences in their habits are known, so that these two species provide excellent material for investigating the influence of the normal experience of the worms on their responses to biologically significant stimuli.

Specimens of *N. diversicolor* in glass tubes were light adapted for 2 hours and then stimulated by a moving shadow which reduced the light instantly from 33·9 to 0·1 ft-candles. Trials were given at 1-minute intervals and the light was obscured for 9 seconds at each trial. After 30 trials, the stimulus was changed to a sudden decrease in light intensity produced by switching off the light for 9 seconds at each trial. Other specimens were treated in the same way except that they were stimulated first by a sudden decrease in light intensity for 30 trials and then by a moving shadow. Specimens of *N. pelagica* were subjected to similar experimental procedures under identical conditions. The results of these experiments are shown in Figs. 6 and 7.

N. diversicolor habituates rapidly to moving shadows (Fig. 6, lower diagram). Worms withdraw only occasionally after the 8th trial and their response is completely extinguished after the 21st trial. No further reactions occur when the stimulus is changed after the 30th trial, to a sudden decrease in light intensity. The worms have a similar rate of habituation to the latter stimulus as to a moving shadow and do not react to a change of stimulus after being habituated to a sudden decrease in light intensity (Fig. 6, upper diagram). Evidently this species does not discriminate between decrease in light intensity produced by shadows and those produced suddenly.

Quite different results are obtained when *N. pelagica* is subjected to this experimental procedure. The rates of habituation to moving shadows and to a sudden decrease are not the same. Habituation to a moving shadow is virtually complete after 3 or 4 trials and the response is totally extinguished after about

15 trials (Fig. 7, lower diagram), but a substantial proportion of the worms react to the first 40 exposures to a sudden decrease in light intensity and some continue to react until the 50th trial (Fig. 7, upper diagram). Not only does this species distinguish between moving shadows and a sudden decrease in light intensity, and habituation to the one take much longer than the
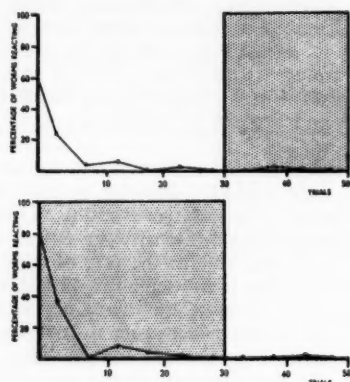

Fig. 6. *Upper diagram:* habituation of *N. diversicolor* to a sudden decrease in light intensity during trials 1-30, followed without a break by 20 trials with a moving shadow. *Lower diagram:* as above, with stimuli presented in the reverse order. Trials given at 1-minute intervals. Observations on 10 worms.
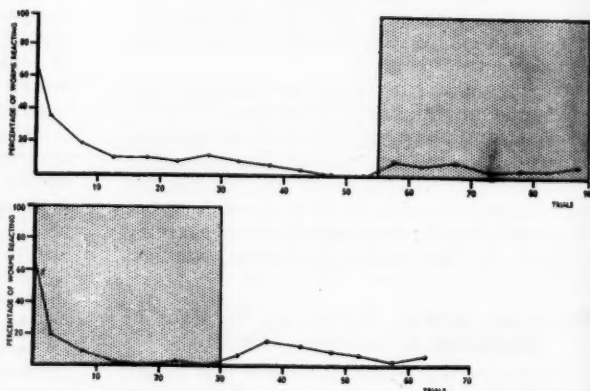

Fig. 7. *Upper diagram:* habituation of *N. pelagica* to a sudden decrease in light intensity during trials 1-55, followed without a break by habituation to a moving shadow. *Lower diagram:* as above, with stimuli presented in the reverse order. Trials given at 1-minute intervals. Observations on 20 worms.

other, but there is also a complex interaction between the two habituation processes (Clark, 1960), with the result that when the worms are habituated to one stimulus, habituation to the other takes longer than usual.

The rate of habituation to moving shadows is clearly not related to the normal experience of the worms. *N. pelagica*, which is constantly exposed to them in its normal environment, habituates to them scarcely more rapidly than *N. diversicolor*, and in view of the individual variability that has been noted in the behaviour of the worms, the slight difference between the habituation rates of the two species is probably not significant. Nor is the habituation rate related to the immediate past experience of individual worms. Specimens of *N. pelagica* investigated a few days after being collected habituate at the same rate to moving shadows as specimens that have been kept in constant, dimly lit aquaria for 5 months before being studied (Fig. 8).
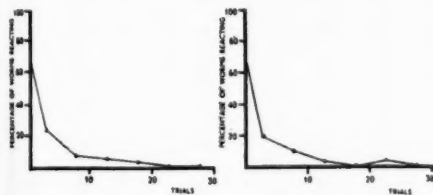
Fig. 8. Habituation of *N. pelagica* to a moving shadow. *Left:* after living in a dimly lit aquarium for 5 months before being tested. *Right:* tested 6 days after being collected. Trials given at 1-minute intervals. Observations on 18 worms.

However, the ability of *N. pelagica* to distinguish a sudden change in light intensity from a moving shadow and its much slower habituation to this stimulus may be related to the normal experience of the worm. If, as I have suggested, a sudden change in light intensity represents a stronger stimulus than a moving shadow, in that the rate of change of light intensity is greater, then it can be concluded that the worm habituates rapidly to slowly moving shadows but not to fast moving ones such as might be caused by a predator. *N. diversicolor* with less experience of shadows, does not distinguish the rate of movement of them, but the fact that it habituates so readily to a change in light intensity, whether fast or slow, appears inexplicable.

Effect of Change of Stimulus on the Reactions of the Worms

It has been known for some time that interruption of a train of stimuli by even a single stronger or different stimulus is sufficient to abolish habituation to the original stimulus in some animals (Humphrey, 1933). Rullier (1948) found that when *Mercierella* has been habituated to both mechanical shock and moving shadows in alternating series of 20 trials with each stimulus, a change of stimulus from one to the other causes a proportion of the worms to react to the new stimulus, though only on the first application of it. This behaviour has some biological advantage, for the worms readily habituate to accidental and repeated stimuli in their environment and yet remain responsive to any change of stimulus. The approach of a predator might be heralded by shadows or mechanical disturbance, but it would certainly involve a different combination of stimuli from those forming the background of sensory experience of the worms.

Similar experiments have been conducted with *Nereis pelagica*. Ten worms were habituated to a moving shadow in a series of trials at 1-minute intervals. The series was interrupted at the 6th, 12th and 18th trials by the interpolation of a mechanical shock (Fig. 9). The worms habituated to the shadow in the three initial trials. Thereafter, except at the 16th trial, the only reactions
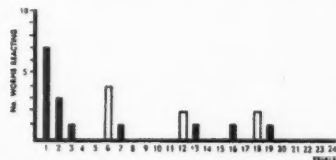

Fig. 9. Effect of interpolating single stimuli by mechanical shock during habituation of *N. pelagica* to a moving shadow. Trials given at 1-minute intervals. *Black blocks:* number of worms responding to a moving shadow; *open blocks:* number responding to mechanical shock. Observations on 10 worms.

to shadows were immediately following stimulation by mechanical shock to which some of the worms also reacted. The total level of reactivity of the worms is not changed by the interpolation of mechanical stimuli, for it has commonly been observed that when *Nereis* is subjected to repeated stimulation by moving shadows, although the worms cease to react regularly after a very few trials, sporadic reactions continue until the 15th-20th trial and sometimes longer.

The effect of the mechanical stimuli is to concentrate the usually sporadic reactions into the trials immediately following those in which mechanical stimulation is given. However, this is effective on the population as a whole and not on individuals, for the worms which react to the mechanical stimulus are not always those that react to the following stimulation by a shadow.

Even the interruption of a train of stimuli by a series of trials with a different stimulus has little effect on the reactions of *Nereis*, as the following experiments show. Worms were completely habituated to a moving shadow in 20 trials at 1-minute intervals. Immediately afterwards they were habituated to mechanical shock in 20 additional trials, and thereafter they were subjected to alternating series of 20 trials with shadows and 20 with mechanical shocks. The results are given in Table II. On the third exposure to a series of mechanical shocks and on the fourth to moving shadows, the worms ceased to react to either stimulus with any regularity. The low level of reactivity that persisted throughout these trials is no more than that which would normally appear after an interval of 20 minutes during which no stimuli are given (Tables III and IV). There is only slight evidence of a resumption of reactions after a change of stimulus and most of the worms are unaffected by the change.

A change of stimulus from one to another of a similar type, but of greater or lesser intensity, has variable effects.

Table II. Effect of Change of Stimulus on the Habituation of *Nereis pelagica* to Shadow and to Mechanical Shock.

Trial	Shadow	Mechanical	Shadow	Mechanical	Shadow	Mechanical	Shadow
1	12	9	3	2	2	0	0
2	4	4	5	1	3	0	2
3	2	4	0	0	0	0	1
4	2	2	3	0	0	0	0
5	0	1	1	1	1	1	0
6	1	0	1	0	1	0	0
7	0	2	1	0	1	0	0
8	1	2	0	0	1	0	0
9	0	0	0	0	1	0	1
10	0	0	1	0	0	0	0
11	0	1	0	0	0	0	
12	0	1	0	0	2	0	
13	1	0	1	0	0	0	
14	0	1	0	0	1	0	
15	1	0	0	0	0	0	
16	0	1	0	0	0	0	
17	0	0	1	0	0	0	
18	2	0	0	0	0	0	
19	0	0	0	0	0	0	
20	0	0	0	0	0	0	

Total of 15 worms in two experiments (results combined).
Each series of 20 trials following the previous series without interruption.

Table III. Reactions of 15 *Nereis pelagica* to Series of 20 Trials at 1-minute Intervals by Moving Shadow, the Series Separated by Periods of 20 Minutes without Stimulation.

Trial no.	Number of worms reacting			
	Series I	Series II	Series III	Series IV
1	11	3	1	2
2	2	1	1	1
3	3	1	1	0
4	1	1	1	0
5	0	0	1	1
6	1	1	0	0
7	1	1	0	0
8	0	0	0	1
9	0	1	0	0
10	0	0	0	0
11	0	1	0	0
12	1	1	1	0
13	0	0	0	0
14	0	0	1	1
15	1	0	0	0
16	0	0	0	0
17	0	0	1	0
18	1	0	0	0
19	1	0	0	0
20	0	0	0	0

(Between Series I–II, II–III, III–IV: *Interval of 20 minutes without stimulation*)

Table IV. Reactions of 15 *Nereis pelagica* to Series of 20 Trials at 1-minute intervals by Mechanical Shock, the Series Separated by Periods of 20 Minutes without Stimulation.

Trial No.	Number of worms reacting			
	Series I	Series II	Series III	Series IV
1	10	2	1	0
2	3	1	0	0
3	2	1	1	0
4	3	0	0	0
5	1	1	0	0
6	0	1	0	0
7	1	0	0	0
8	0	1	0	1
9	1	0	0	1
10	0	0	0	0
11	2	0	1	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	1	0	0	1
18	1	0	0	0
19	1	1	0	0
20	0	0	0	0

(Between Series I–II, II–III, III–IV: *Interval of 20 minutes without stimulation*)

It has already been shown that a change of stimulus from a sudden decrease in light intensity to a moving shadow (stronger to weaker stimulus) delays habituation to the latter, while a change of stimulus in the reverse direction has complex effects on the subsequent habituation to a sudden decrease in light intensity, as well as delaying it (Clark, 1960, Figs. 10 and 11).

No such complex effects are found when the worms are first partly habituated to a combined stimulus of mechanical shock and sudden decrease in light intensity, and then subjected to one or other of the constituent stimuli (Fig. 10). Habituation to the latter proceeds at about the normal rate.

Persistence of Habituation

We have not so far considered the length of time an animal remains partially or totally habituated after the source of stimulation has been withdrawn. During this period the worms are less reactive and so more vulnerable to attack by a predator.

There is a slight return of reactiveness even as little as 20 minutes after being habituated to mechanical shock or a moving shadow, though only a small number of worms react (Tables III and IV). At longer intervals after a train of stimuli to which the worms have become habituated, a greater proportion react to renewed
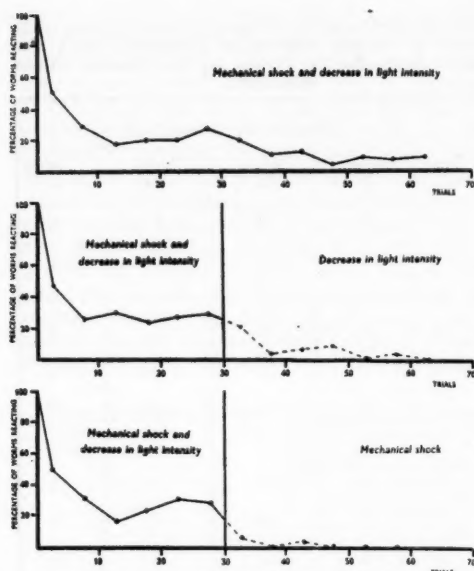
Fig. 10. Effect of decreasing the strength and complexity of the stimulus on the habituation rate of *N. pelagica*. *Top:* simultaneous presentation of mechanical shock and sudden decrease in light intensity; *middle:* stimulation by mechanical shock and decrease in light intensity during the first 30 trials, then by sudden decrease in light intensity only; *bottom:* stimulation by mechanical shock and decrease in light intensity during the first 30 trials, then by mechanical shock only. Trials given at 1-minute intervals. Observations on 23 worms.

stimulation and the number of stimuli necessary to bring about complete habituation increases (Clark, 1960, Figs. 6 and 7).

It was previously shown (Clark, 1960) that the persistence of habituation of *N. pelagica* is a

function of the frequency of stimulation. Worms stimulated by a sudden increase of light intensity at intervals of 3 minutes do not recover completely from habituation until about 17 hours have passed. Worms stimulated in a similar manner at $\frac{1}{2}$-minute intervals recover within $5\frac{1}{2}$ hours.

The effect of varying the number of trials on the persistence of habituation to a moving shadow is shown in Table V. In the first experiment worms were exposed to 20 trials at 1-minute intervals by which time they are completely habituated. The same individuals were tested at intervals of 20 minutes, 40 minutes, 1 hour 40 minutes, and 2 hours 40 minutes after the end of previous series of 20 trials in which they had been habituated. Although after 40 minutes they show a substantial loss of habituation, recovery from habituation is not complete until nearly 3 hours have passed. Worms habituated to a moving shadow by exposing them to 50 trials at 1-minute intervals in each test do not recover fully for 7 hours.

The nature of the stimulus, although it determines the number of stimuli necessary to produce habituation, has no direct influence on the persistence of habituation. Habituation to mechanical shock and to moving shadows are quite independent processes. If the worms are habituated to mechanical shock in 20 trials at 1 minute intervals, full recovery takes about 3 hours, which is the same as the length of time necessary for recovery from habituation to a moving shadow established by exposing the worms to the same number of trials at the same frequency (Table V).

Translated into biological terms, these results mean that if the worm in its normal environment

Table V. Influence of Frequency and Number of Stimuli, and the Nature of the Stimulus on the Persistence of Habituation of *Nereis pelagica*.

Nature of stimulus	Number of trials	Interval between trials	Duration of habituation
Sudden decrease in light intensity	55	3 min.	17 hr.
Sudden decrease in light intensity	55	$\frac{1}{2}$ min.	$5\frac{1}{2}$ hr.
Moving shadow	55	1 min.	7 hr.
Moving shadow	20	1 min.	3 hr.
Mechanical shock	20	1 min.	3 hr.
Moving shadow	20	1 min.	3 hr.

is subjected to repeated but unreinforced stimulation it will become habituated and cease to react to the stimulus after a period, the length of which depends upon the nature of the stimulus. If stimulation then ceases the worms remain insensitive to the stimulus for a time. The progress of recovery from habituation depends upon the number and frequency of stimuli they have previously received. Within a relatively short period a substantial proportion of the worms react to a renewed application of the stimulus, but they habituate very quickly to it again. It is only after a fairly long period that they recover totally from habituation. Under natural conditions, *Nereis* must be exposed to such stimuli as moving shadows and mechanical shocks for much longer periods than have been employed in these experiments. The worms are therefore likely always to be in a state of partial habituation. Total habituation will follow very rapidly upon the onset of renewed stimulation.

Discussion

The withdrawal reflex of tubicolous polychaetes has been assumed to be a defence mechanism against predators and there seems no reason to dispute this assumption. The predators of *Nereis* include bottom-feeding fish, shore birds and, probably, large crustaceans. Stomach analyses of many bottom-feeding fish indicates that polychaetes figure largely in their diet. The analyses are rarely detailed enough to show whether nereids are taken in quantity, but various species of *Nereis* including *N. pelagica*, have been identified in the gut of cod, haddock, flounder and plaice (McIntosh, 1908-1910; Franz, in Wimpenny, 1953) and *Nereis* is used as bait in many parts of the world for a great variety of fish. A number of shore birds have been recorded as feeding upon marine worms and *Nereis* has been identified in the diet of godwit, curlew, dunlin, sandpipers, plovers, some ducks, terns and gulls (Collinge, 1924-27; Witherby *et al.*, 1938-41). The larger crustaceans such as crabs certainly eat polychaetes since they are carnivorous (Yonge, 1954) and polychaetes represent one of the most readily available foods. *Nereis pelagica* living in glass tubes are captured and eaten by crabs (*Carcinus maenas*) in the aquarium.

All these predators, so far as their hunting methods are known, create mechanical disturbances of one sort or another, and they cast shadows. *Nereis pelagica* lives in an environment in which it must be exposed almost continuously to accidental stimuli of the same kind, and its response to them suggests that it is not unduly distracted by them.

The usual reaction of the worms to such stimuli can be summarized as follows. On receipt of a stimulus after a period of constant conditions, most of the worms contract. Not all of them do so and an average of 10-15 per cent. (Fig. 4), and in some experiments, as many as half, or even more, of the specimens fail to contract on first being stimulated. The worms remain contracted for a variable period, but most extend again within 1 minute (Fig. 3) and react only intermittently to subsequent stimulations; the number that react to 2 or more consecutive stimuli is very small (Fig. 4). An extreme variability of the behaviour of individuals and also of the same individuals on different occasions is a characteristic of *Nereis* (Figs. 1 and 2) and the habituation curves that have been derived give an indication of the proportion of worms in a population that react after a number of repeated stimulations, but are no guide to the behaviour of individual worms. A population of worms habituates most slowly to a sudden increase in light intensity and to a combination of mechanical shock and a sudden decrease in light intensity (Table I), but most worms cease to react regularly after the initial exposure to even these stimuli. The rate of habituation is not influenced by a long experience of constant conditions (Fig. 8), nor, surprisingly, does a species such as *N. diversicolor*, which rarely if ever experiences shadows or mechanical shocks from wave action in its natural environment, habituates more slowly than *N. pelagica* (Figs. 6 and 7).

Such rapid habituation prevents the interruption of maintenance activities of the worms by accidental stimulation, though it leaves the worms vulnerable to attack by predators. A change in the nature of the stimulus only slightly increases the reactivity of the worms (Table II and Fig. 9), and intensification of the stimulus has at most a partial and delayed effect (Clark, 1960, Figs. 10 and 11). Furthermore, once the animals have become habituated to a given stimulus, recovery is slow (Table V) and although after a fairly short interval, half the worms respond to a renewed application of the stimulus, they become habituated to it again very much more quickly than before (Clark, 1960, Figs. 6 and 7). It is difficult to escape the conclusion that in its natural environment, *Nereis pelagica* is almost always in a state of partial or total habituation to a wide variety of stimuli.

Under these conditions such simple stimuli as shadows and mechanical disturbances can hardly serve as token signals of the approach of a predator, which Nicol (1950) suggested might be the case in *Branchiomma*. Recognition of a predator's approach is therefore likely to be by means of a more complex configuration of stimuli, and, in support of this conclusion, it may be recalled that habituation to multiple stimuli is slower than to the constituent ones (Fig. 10). Recognition of a situation by a complex of its characteristic features rather than by a token, representative stimulus, demands a highly organized sensory and central nervous system and this the Nereidae have, compared with sabellids. The withdrawal reflex is widespread among tubicolous, worm-like animals and generally there is a through-conduction pathway in the nerve cord, in the form of giant axons, by which this response is intermediated. In some of the more highly specialized tubicolous polychaetes, and especially in the Sabellidae, these axons reach an enormous size and occupy a very large proportion of the nervous system (review by Nicol, 1948). Consequent upon this high degree of morphological specialization of the nervous system, the sabellids have only a meagre sensory equipment and a very limited range of behaviour. In *Nereis*, on the other hand, the giant axons are of more modest dimensions (Bullock, 1945; Smith, 1957) and the remaining part of the nervous system is complicated and highly organized (Holmgren, 1916; Hanström, 1928; Smith, 1957), the sensory system is complicated, and the worms are more versatile and more discriminating in their behaviour.

Summary

1. Individual *Nereis pelagica* differ from one another and on different occasions in their reactivity to repeated stimuli. The majority of worms react only to the first stimulus of a series, recover from it within 1 minute and react only sporadically to subsequent stimuli.

2. The rate of habituation of *N. pelagica* to repeated stimuli which may serve as warnings of approach of a predator, is inversely related to the biological significance and urgency of the stimulus.

3. The sensitivity of *N. pelagica* to shadows is not influenced by previous experience, nor does *N. diversicolor*, which normally has no experience of shadows, habituate to them less rapidly than *N. pelagica*, although there is evidence that

the latter species distinguishes between fast and slow charges in light intensity, whereas *N. diversicolor* does not.

4. Interruption of a series of repeated stimuli by a different stimulus, or alternation of series of different stimuli does not increase the reactivity of *Nereis*.

5. Recovery from habituation is slow and depends upon the frequency and number of stimuli previously given rather than upon the nature of the stimulus.

6. It is concluded that *N. pelagica* does not recognize the approach of a predator by a simple token stimulus as some sabellids may do. The worm is thought to be in a state of partial or total habituation to the simple stimuli which are almost constantly represented in its normal environment, and that recognition of a predator's approach is by a complex of stimuli.

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BRIEF COMMUNICATIONS

EFFECT OF VISUAL AND VOCAL STIMULI ON BREEDING IN THE BUDGERIGAR (*Melopsitacus undulatus*)

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Introduction

In birds the importance of light for the stimulation of the gonads has been generally accepted. Recent reviews have critically considered the evidence for such a concept (Benoit & Assenmacher, 1959; Hammond, 1954; Wolfson, 1959; Yeates, 1954). The importance of other factors such as availability of food and nesting material has been recognised (Marshall, 1955). Until recently the following quotation from Burger (1949): "No experiment thus far devised has proven that any external stimulus, *operating in darkness*, induces precocious spermatogenesis" seemed to be supported by the available evidence. However, Vaugien (1951), carried out experiments with Budgerigars (*Melopsitacus undulatus*) which indicated that the sound of other Budgerigars courting in the aviary stimulated the development of ovarian follicles and oviducts. Vaugien (1952, 1953) also contended that male and female gonads developed sooner in darkness than under light. Marshall & Serventy (1958) confirmed the observation that spermatogenesis could occur in darkness, using Zebra Finches (*Peophila castanotus*), but disagreed with the contention that the development was faster in darkness.

Under the conditions of Vaugien's experiment (1951) birds of only one sex (females) were placed in dark boxes in the aviary. Under these conditions the possible stimulation by the mate was eliminated. That such stimulation can be important has been shown by Polikarpova (1940) for House Sparrows (*Passer domesticus*) and by Burger (1942) for Starlings (*Sturnus vulgaris*). The experiment reported here was designed to test the relative importance of auditory and visual stimuli for the stimulation of the gonads of pairs of Budgerigars kept in individual cages.

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Methods and Materials

Birds. One and two year old virgin Budgerigars, which had been able to see and hear members of both sexes were used‡. Male and female Budgerigars were paired together at random. The latter were supplied with a nest box, and food and water *ad libitum*. For Treatment A, B, C (see below) cages were arranged in rows of 4. Each row received the same treatment. All these cages were housed in a heated, windowless shed where artificial, fluorescent light was provided from 8 a.m. to 5 p.m. Before the experiment all birds underwent laparotomy and the size of the gonads estimated. Only birds with obviously inactive gonads were used.

Treatments. A. Each pair could hear and see other pairs;

B. The pairs could not see other pairs because of partitions placed in the cages. Each cage was provided with 4 mirrors, to serve as a control for treatment D;

C. Like B, but no mirrors;

D. Each cage was placed in a separate room so that the birds could not hear other pairs. Each cage contained 4 mirrors to stimulate the condition of "seeing but not hearing other pairs." The idea of using a mirror came from the experiment by Matthews (1939) in which it was found that placing a mirror induced ovulation and laying in pigeons as did seeing another pigeon in another cage;

E. As D, but no mirrors.

It should be pointed out that since these cages for Treatment D and E had to be placed in laboratory rooms and offices, the conditions were not exactly the same as for treatments A, B and C. For instance in Treatment D and E daylight could reach the cages, the temperature was more variable because steam pressure was lowered during week-ends. However, there was also variation within Treatments D and E. In some rooms there was more activity than others.

In spite of these differences the results in the Treatments D and E were consistent and it was judged therefore, that these results were not invalidated by this somewhat less than ideal experimental set up.

Measurements. The birds were killed after thirty-five days on the experiment. Gonads and oviducts were fixed immediately in Bouin's solution and weighed later. The testes were dehydrated, embedded in paraffin, sectioned at 8 micra and stained with hematoxylin eosin. The stage of spermatogenesis was scored by the system used by van Tienhoven *et al* (1956).

Analysis. Data were analysed by analysis of variance (Snedecor, 1956).

Results and Discussion

The results obtained are presented in Table I and the statistical analysis of the results in Table II. No statistical analysis was carried out for ovary weight and oviduct weight, as the differences between Groups A, B and C, when compared with D and E were of such magnitude that statistical analysis was superfluous. On the other hand oviduct weight in Group A was almost certainly lower than that in Group C because some birds had laid their clutch and the oviduct was regressing. Ovary weight comparisons between Groups A, B and C were not meaningful since some birds had just ovulated when killed and thus a lighter ovary would be expected.

The data on spermatogenetic activity were not analysed statistically because the large difference between A, B and C versus D and E made it unnecessary, whereas there was no difference between A, B and C and only a very small difference between D and E.

The data, though somewhat limited in extent, permit in our judgment, the following conclusions:

1. Under conditions in which feed and water are available *ad libitum*, and when a nest box is provided, vocalizations of other pairs of the same

Table I. The Relative Effect of Visual and Auditory Stimuli on the Weight of Reproduction Organs, Stage of Spermatotogenesis, Number of Eggs Laid and Interval to First Egg in Budgerigars.

Treatment	Pairs	Testes wt.	Spermatog. stage	Ovary wt.	Oviduct wt.	Eggs laid	Days to first egg
	No.	mg.		mg.	mg.	No.	No.
A) Hear and see	4	299·7	7	535·9	429·0	4·0	24·7
B) Hear, mirrors	3	280·9	7	258·1	646·0	0·6	32·0
C) Hear	4	349·7	7	216·3	1129·3	2·5	26·3
D) Solitary, mirrors	3	63·3	3·7	27·2	24·0	0	—
E) Solitary	4	36·3	2·8	17·8	25·7	0	—

Table II. Analysis of Variance for Testes Weight, Number of Eggs Laid and Days to First Egg Data from Table I. F Values and Probability Levels.

Source		Characteristic					
		Testes wt.		Number of eggs		Days to first egg	
	df	F	P	F	P	F	P
A, B, C versus D, E	1	37·09	<0·001	8·51	<0·0025	17·43	<0·005
A versus B	1	<1	>0·20	7·00	<0·0025	7·84	<0·05
A, B versus C	1	1·08	>0·20	<1	>0·20	1·95	>0·05
D versus E	1	<1	>0·20				
Error	13	8753·41*	——	3·205*	——	400·75*	——

*Error mean square.

species enhance the development of the gonads of both sexes of Budgerigars.

2. Under conditions as mentioned above the presence of mirrors inhibits the rate of development of the ovary, as measured by interval to first egg and number of eggs laid within the duration of the experiment. Under the same conditions no effect on testes development could be detected. However, only the initial and final testes size, when all testes were developed, were measured. Thus, a slight delay in testicular development, if present, would not have been detected in this experiment.

No explanation can be given for this effect of mirrors on female gonad development. The stimulation of gonad development by vocalisations from other birds may of course be important in a species that is as gregarious as the Budgerigar.

Summary

Under conditions in which food, water and a nest box were provided to caged pairs of Budgerigars, vocalizations from other pairs stimulated male and female gonad development. Under these conditions the presence of four mirrors in a cage had an inhibitory effect on the rate of development of the ovary but not of the testis.

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A NOTE ON THE EFFECT OF EARLY BLINDNESS ON SEXUAL AROUSAL IN THE MALE RAT

BY M. GREENBAUM AND D. L. GUNBERG

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In the course of a larger study on the effects of neonatal trauma on later adaptive behaviour in the rat, one group of animals was blinded in infancy and subsequently examined in a mating situation and also in a procedure designed to reveal proneness to somatic stress reactions. The results indicated that it would be of value to report separately on the effects of blindness on sexual arousal.

Several earlier studies (Beach, 1942; Stone, 1922) have indicated that the blinding of adult sexually experienced male rats does not interfere with their mating patterns and skills. Some sexually *inexperienced* males, however, may show a decrement in copulatory reactions following enucleation. Presumably vision plays a supporting role in the initial stages of copulatory experience and its absence may interfere with arousal in animals who have a high threshold for sexual excitation (Beach, 1942).

Method

In the present study the eyes of 11 male Long-Evans rats were removed under ether anaesthesia on the 15th postnatal day. The rats were returned to their mothers and remained with them until weaning on the 24th day of life when they were separated into cages of 3 to 4 animals each. Eleven control animals were simply weaned at 24 days of age and raised under similar conditions as the blinded group. All animals were maintained on diets of chow and water (*ad libitum*).

Sex tests were begun when the animals were 98 to 101 days of age. Males were transferred to individual metal cages, 16 in. × 9 in. × 7 in., forty-eight hours prior to the evening of the test. A receptive female in the œstrous or pro-œstrous phase as determined by vaginal smear and confirmed by behaviour observations was placed with the male and allowed to remain until the male demonstrated a mounting response (rear mounting with palpation and pelvic thrusts). The test was discontinued if mounting did not occur within 10 minutes. Non-mounters were given an additional test 24 hours later.

Results

Sixty-three per cent. of the enucleated group demonstrated arousal to the point of mounting whereas 100 per cent. of the controls attained the mounting response. The mean latency to time of mount (1009 seconds vs. 104 seconds for the controls) also disclosed the deficit in arousal of the blinded group. Since 4 animals in the enucleated group did not mount within the 1200 seconds observation period the mean and S.D. of the latencies for that group were estimated for a singly censored sample (Sarhan & Greenberg, 1958).

The comparison between groups was made by applying the Kolmogorov-Smirnov test (Tate & Clelland, 1957) to the mount latency cumulative percentage curves and analysing them for differences in distribution. In this analysis the curve for the blinded group differed significantly ($P = \cdot 01$) from the untreated controls. The blinded group showed significantly diminished arousal. Since there were no differences between these groups in testicular or seminal vesicle weight it does not appear that a physiologic deficiency can account for the diminished arousal.

Table I. Percentage of Mounters and Mount Latencies, for Blinded and Control Animals.

	% Mounters	Mount latency (sec.)	
		Mean	S.D.
Untreated controls n=11	100	104·27	83·34
Blinded n=11	63·3	1008·88*	570·71

*Estimated with order statistics for the case of singly censored samples.

Discussion

These results provide additional evidence to that accumlated by Beach (1942) which indicates that some animals suffer copulatory deficiencies secondary to blindness. It should be noted, however, that Beach's animals were blinded

when relatively mature while ours had no demonstrable visual experiences because of their early enucleation. Thus, the length of time of visual deprivation may not be a significant factor provided the animals are sexually inexperienced at the time of enucleation. Since the control animals did not receive sham operations it has not been established that the actual operative process of enucleation and disturbances induced by this procedure were not significant factors in the latter behaviour. In this sense some qualification must be placed on the present findings.

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THE DIVING AND SURFACE-WALKING BEHAVIOUR OF *Dolomedes triton sexpunctatus* (Araneida : Pisauridae)

BY W. H. McALISTER

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Introduction

Nursery web spiders of the genus *Dolomedes* are common components of the shoreline and littoral zones of lentic freshwater communities throughout most of the United States. Correlated with their amphibious mode of life these araneids are capable of running over and diving beneath the surface of the water. The present report is the result of a study of the diving and surface-walking behaviour of *D. triton sexpunctatus* Hentz, which is considered the most aquatic species in the genus (Bishop, 1924).

Methods

Three types of submergence tests were conducted in the laboratory. In the first of these $3\frac{1}{4}$ in. of pond water was placed in a gallon jar. A paper hand towel was crumpled and put into the water to serve as a support for spiders which attempted to dive. A spider was introduced onto the surface of the water and prodded from above for 30 seconds, or until it dived. If the spider dived the duration of submergence was determined with a stopwatch. A second test was identical with the first except the paper towel was removed. The final test involved placing a spider in a glass vial and covering the mouth of the vial with cheesecloth. The vial was then submerged in pond water and the length of time the spider remained responsive to prodding with a needle was recorded. These three tests were respectively designed to reveal: (*a*) the usual duration of a submergence initiated by provocation and terminated when the spider voluntarily arose to the surface; (*b*) the ability of a spider to dive without the benefit of a solid support; (*c*) the maximal time an individual could endure submergence.

The manner of diving was observed in the field and laboratory and is briefly described.

The surface tension of distilled water was progressively lowered in a series of 25 ml. dishes. Surface tension depression was achieved by introduction of a detergent. The water temperature was 28°C. Spiders were placed on the surfaces of the test solutions in order to deter-

mine the critical surface tension at which the animals fell through the surface film.

Results

Twenty-four individuals were subjected to voluntary submergence tests with a solid support. All but one specimen dived readily. The exception curled into an immobile compact ball on the surface of the water where it remained for 3·2 minutes. The average dive duration was 7·6 minutes. One individual remained submerged for 27 minutes (the maximum recorded voluntary duration) when observations were necessarily ceased. There was no significant difference in the duration of dives made by juveniles and those made by adults.

Eight *Dolomedes* were subjected to prodding with no support from which to dive. None of the spiders could break the surface film until the side of the container was used as a support. Three specimens dived by utilizing the jar wall, and these individuals beat the legs wildly as they swam to the bottom. They then popped to the surface where two managed to break through the surface film. The third spider was unable to emerge and moved about beneath the surface film until offered a support on which it immediately climbed out. The spiders are apparently incapable of entering the water without a solid support, and once submerged they experience difficulty in getting back onto the surface without some solid object from which to push through the surface film. In nature emergent vegetation apparently serves as the chief support for diving spiders.

Six *D. t. sexpunctatus* were placed in vials to determine the maximum duration of submergence before death occurred. Seven additional spiders representing five families were also tested, and the results are presented in Table I. These data indicate that *D. t. sexpunctatus* is physiologically capable of surviving prolonged submergence. This result is to be expected since such a capacity has probably been improved in the pisaurids by natural selection. One lycosid (*A. littoralis*) frequents the shoreline of lentic

Table I. Araneids were placed in a Vial and Submerged in Pond Water until they no longer moved when Prodded with a Needle. Duration results indicate the maximum time each individual remained responsive to the needle.

Species	Maximum duration of response to prodding with needle (minutes)
Dolomedes t. sexpunctatus (*Pisauridae*)	26, 81, 109, 126, 136, 184
Arctosa littoralis (*Lycosidae*)	45, 73
Lycosa sp. (*Lycosidae*)	16
Lycosa sp. (*Salticidae*)	9·3
Agalenopsis sp. (*Agalenidae*)	13·8
Scytodes intricata (*Scytodidae*)	12
Neoscona oaxacensis (*Argiopidae*)	13·8

Table II. Individuals of *Dolomedes t. sexpunctatus* were Placed on the Surface of Water, the Surface Tension of which had been Lowered by the Introduction of Detergent. The surface tension of pond and marsh water is included for comparison. A surface tension below 40 dynes/cm. is not to be expected in nature.

Test solution	Surface tension (dynes/cm)	Reaction by spiders
1 (Distilled water)	72·8	Easily remained on surface;
2	48·4	Negative meniscus of tarsi more evident;
3	42·1	Sternum and palps in water contact;
4	39·9	Spiders flat on surface;
5	38·8	Spiders immediately fell through surface;
Farm pond	68·8	
Marsh	59·4	

communities and occasionally dives beneath the water when disturbed. Its resistance to submergence is relatively high (45-73 minutes). A more terrestrial lycosid (*Lycosa* sp.) died after remaining under water only 16 minutes. A salticid, which leads a terrestrial and arboreal existence survived only 9·3 minutes.

When it dives, *D. t. sexpunctatus* usually does so head first, suddenly, and it may run down a submerged support several inches before it stops. Usually, however, specimens stop within an inch of the surface. While under water these animals usually remain motionless. They then ordinarily turn suddenly and run up the support to immediately break through the surface film. The surfacing movement is abrupt. A single specimen of *D. urinator* exhibited a different emergence pattern. This spider, after a voluntary dive of 5·4 minutes, slowly ascended and protruded the ocular quadrangle and first pair of legs above the surface. It remained in this position for 7·0 minutes, when it was necessarily disturbed.

Submerged individuals of both *D. t. sexpunctatus* and *D. urinator* were observed to swim short distances in both vertical and oblique planes. This is accomplished by movements of the ambulatory legs. No spider voluntarily swam from a submerged support.

Results of the surface tension experiment are given in Table II. Three individuals *D. t. sexpunctatus* weighing 0·8, 0·4, and −0·1 grammes were able to maintain their position on all test solutions except that one in which the surface

tension was reduced to 38·8 dynes/cm. Welch (1952) mentioned that at a surface tension of 50 dynes/cm. a water strider (*Gerridae*) fell through the surface film, and a water measurer (*Hydrometridae*) flattened itself to remain afloat. It is doubtful if the surface tension of natural waters ordinarily falls below 45-50 dynes/cm. Except under extreme conditions, therefore, reduced surface tension is probably no problem to *Dolomedes* in nature.

Conclusions

Dolomedes triton sexpunctatus leads a semi-aquatic existence in the shoreline zone of freshwater communities. Its surface-walking and diving behaviour allow it to temporarily enter the supraneuston and periphyton habitat niches.

In the laboratory the dives of this pisaurid usually last from four to nine minutes, although one spider voluntarily remained submerged for almost half an hour. Individuals require a solid support to push themselves into and out of the surface film and a support is also used as an anchor to which the spiders cling while submerged. Compulsory submergence tests conducted in the laboratory indicate that *D. t. sexpunctatus* is physiologically adapted to remaining underwater. It is evident that aside from

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the physiological capability of remaining under-water, a correlative psychological pattern has developed in these spiders to allow them to efficiently utilize their capacity.

Laboratory experiment suggests that *D. t. sexpunctatus* is hindered in its movements over the surface of water when the surface tension drops below 40 dynes/cm. This critical value is below that ordinarily encountered in nature.

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PROCEEDINGS OF THE SECTION OF ANIMAL BEHAVIOR AND SOCIOBIOLOGY (ECOLOGICAL SOCIETY OF AMERICA AND THE AMERICAN SOCIETY OF ZOOLOGISTS)

SYMPOSIUM ON ANIMAL BEHAVIOUR

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STUDIES IN OLFACTORY ACUITY. 4. RELATIVE DETECTABILITY OF n-ALIPHATIC ACIDS BY THE DOG

By D. G. MOULTON*, E. H. ASHTON and J. T. EAYRS†

Department of Anatomy, University of Birmingham

The ability of the dog to respond to cues which man does not normally detect has been widely exploited (as in tracking and truffle hunting) and has led to the belief that its sense of smell is far superior to that of man. This view would seem in accord with the extensive nature of the dog's olfactory receptor surface which, according to Adrian (1956), is as closely packed with receptors as is the retina and is, at the same time, much larger than those of its eyes and ears combined. It is important, however, to distinguish between absolute olfactory acuity and the ability to discriminate between different odours. Thus, although the numerous field trials of Dutch and German workers on tracking ability suggest that the dog possesses highly developed powers of olfactory discrimination (see reviews by Buytendijk, 1935; Warner, 1936; McCartney, 1951), they do not necessarily imply an absolute olfactory acuity greatly in excess of man's. In fact Neuhaus (1953) claims that a man can easily detect perspiration on a sheet of paper over which a person wearing shoes has walked at normal speed, and he has estimated that the amount of butyric acid deposited is considerably more than is required to elicit a threshold response.

While data derived from laboratory experiments might be expected to provide more reliable information about olfactory acuity, published values show divergences which are probably among the most extreme reported for any sensory function. Thus Neuhaus (1953) concludes that the dog can detect fatty acids in concentrations of one million to one thousand million times lower than can man, whilst Niccolini (1954) gives data which support his claim that canine and human olfactory thresholds are similar. Consistent with this view is the failure of Becker, Markee & King (1957) to train dogs to respond to concentrations of oil of cloves below those detectable by man. Studies by Binet & Passy (1895), and Buytendijk (1921) have little significance in this context since although they give data relating to the detectability of fatty acids by the dog which lie within,

or close to, the range of published human thresholds these are merely concentrations to which the dogs responded rather than absolute thresholds.

There are major differences in the techniques used by these workers, and the amount of training given to a dog (Krushinsky, Chuvaev & Volkind, 1946; Neuhaus, 1953) as well as the biological significance of the odorants used (Heitzenroeder, 1913; Seffrin, 1915; Adrian, 1954) have been shown to have an important influence on the level of olfactory sensitivity recorded. However, in attempting to account for discrepancies a further factor seems especially significant. With one exception the above workers fail to state either that no observer was present during trials or that any observer present was unaware of the position of the test substance. Thus the possibility exists that some cue could have passed from observer to dog which could have influenced the results. The reality of this source of bias is illustrated by the one exception. Using a problem box situation Becker, Markee & King (1957) found that significantly higher scores were obtained when the handler knew the position of the odorant than when he did not.

It seems, then, that there is little reliable evidence on the subject of the dog's olfactory acuity and that such evidence as is available is in any case conflicting. More precise data have recently been obtained for the rat using carefully controlled experimental conditions (Eayrs & Moulton, 1960; Moulton & Eayrs, 1960; Moulton, 1960) and in the present study similar techniques have been applied to an assessment of the relative detectability of the first eight monobasic n-aliphatic acids by the dog. A preliminary account of part of this work has already appeared (Ashton, Eayrs & Moulton, 1957).

Materials

The experiments were carried out in a wooden hut 12 feet square which was partitioned into three rooms: a main experimental room; a room for the handler and the dog and a room for the supervisor (Fig. 1). Since the doors allowed complete isolation of each room the

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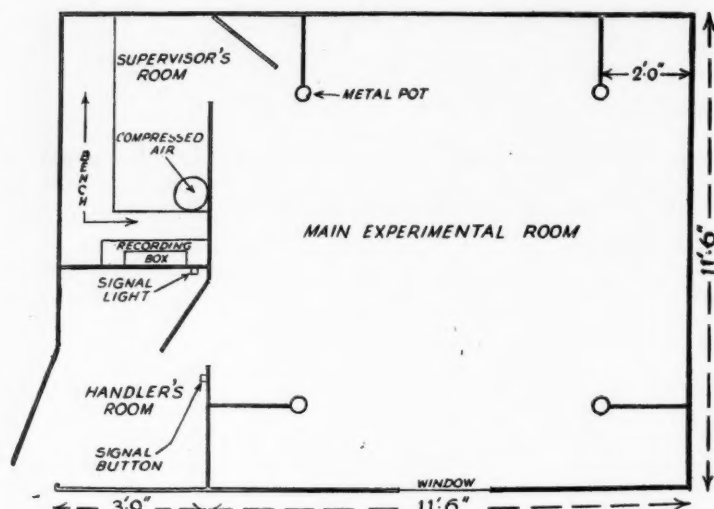†Henry Head Research Fellow of the Royal Society.

Fig. 1. Plan of experimental hut.

open during trials, together with a fan in the roof, allowed a constant circulation of fresh air and so minimised the possible effects of a general contamination with odour.

Near each corner of the main experimental room was a station, or unit of equipment, consisting of a small metal pot on the floor and a meat container, bell push and signal light on the wall. Their relative positions can be seen in the perspective diagram of the hut (Fig. 2). Each pot, which was a length of metal tubing with a metal base and a wire gauze lid, was screwed to a floor socket

supervisor could set out an experiment without being able to see the dog, whilst the handler and dog were unable to watch the supervisor. Thus the handler could know neither the arrangement of the test substances nor the correctness of the dog's response until it had been made. A window in the main experimental room, which was kept

and could be easily removed for cleaning. A polythene tube, connecting through copper and rubber tubing to a cylinder of compressed air in the supervisor's room, was inserted near the base of each pot and allowed a blast of air to be injected between trials. During experiments a small metal crucible containing about one ml. of

the test liquid was placed in each pot. Fig. 3 shows a cross section of a pot with crucible in place.

All signal lights and bell pushes were connected to a control box in the supervisor's room and switches on this box could be set so that each bulb lit up or did not light up when its own bell push was operated. Flag indicators incorporated in the electric circuit showed the supervisor the position of each bell push that was pressed.

The fatty acids used were obtained from British Drug Houses Limited and were diluted with freshly distilled water. A new set of glass-stoppered
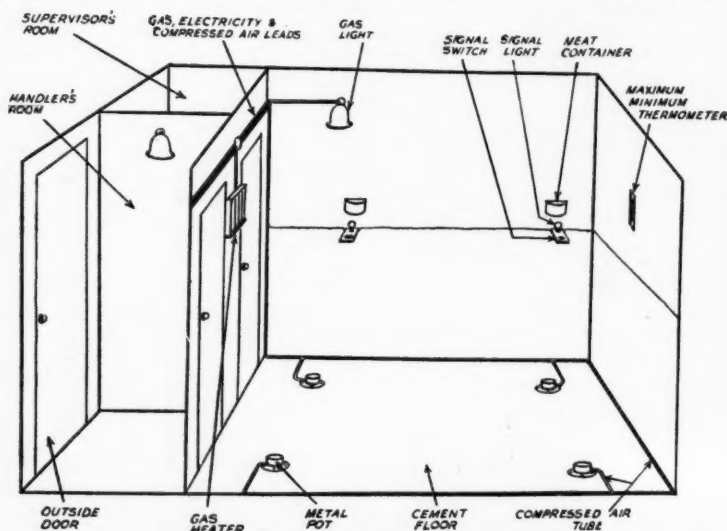

Fig. 2. Perspective diagram of experimental hut.

WIRE GAUZE

METAL CYLINDER TO SUPPORT GAUZE

METAL POT (SCREWED TO BASE)

POLYTHENE TUBE (COMPRESSED AIR)

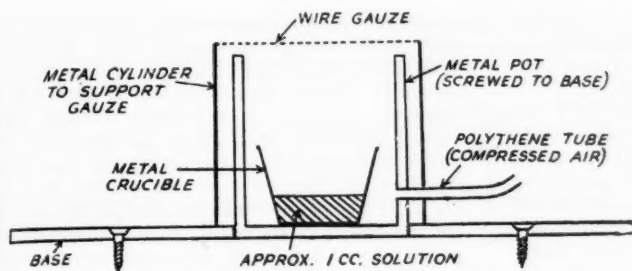METAL CRUCIBLE

BASE

APPROX. I CC. SOLUTION

Fig. 3. Sectional view of crucible and pot.

glass bottles was used for every series of dilutions of an acid.

The two subjects of the experiment were cross-bred Labradors, one being a spayed bitch (Venus) and the other a dog (Dick). Their food intake was restricted to a level which ensured adequate motivation during trials.

Methods

1. Training and Experimental Procedure

During preliminary training the dogs were taught to sniff at each pot in turn, to sit at those containing acid and to pass by those which did not. At the beginning of each trial, with the handler and dog confined in their room, crucibles containing either acid or distilled water ("blanks") were placed in each of the four pots by the supervisor who then returned to his room and set the control switches so that only the signal lights at stations housing acid-containing crucibles were illuminated when their bell pushes were operated. He then removed any trace of acid vapour remaining from a previous trial by releasing a blast of compressed air. This took about 10 seconds, at the end of which a signal was given to the handler, by means of a signal light, to begin the trial.

When a dog sat at a station the handler determined whether or not the response was correct by pressing the appropriate bell push. The appearance of a light informed the handler that the pot contained an acid and the dog was given a reward of meat from the container on the wall. Each container, whether associated with positive or negative stations, contained meat. If no light appeared the pot contained a blank and the dog was given a light slap. The handler indicated the end of each trial by operating a signal-light in the supervisor's room. Thus all communication between handler and supervisor was restricted to signal exchanges.

The dog's score was derived from an inspection of any indicator flags that appeared in the control box during a trial, indicating where the dog had sat. A mark was awarded for every blank pot which the dog had passed and every acid pot at which it sat. When the dog reached a stable performance on a concentrated solution of a given acid a series of dilutions of the acid was prepared, each dilution being half the concentration of that used previously. (The method was essentially similar to that described by Beck, Kruger & Calabresi, 1954). A preliminary estimate of threshold concentration was then made which enabled an appropriate series of dilutions to be selected for an experiment. The normality of the strongest solution was determined by titration.

Each day all crucibles, pots, lids, pipettes and tongs used in experiments were boiled in distilled water and dried out in an oven. Crucibles and pipettes which had contained acid were not separated from those which had contained distilled water.

2. Statistical Design

There are sixteen possible ways of combining blank and acid-containing crucibles in four pots, and in a unit comprising each of all possible combinations, equal numbers of the two types of crucible will occur. By the method of scoring used, a dog could attain a maximum of 64 points in one unit although half of these points could be obtained by choosing the pots at random.

In order to minimise the influence of day-to-day fluctuations in performance a balanced incomplete block design was employed, involving 7-9 concentrations of an acid worked in blocks of three units. In the final analysis the aggregated scores for each concentration converted to percentage successful choices, were subjected to the probit transformation (Finney, 1952), which gave an adequate correction to the sigmoid curve formed by plotting this data against the log concentration of an acid. The midpoint of the probit regression lines for each acid was therefore taken as a measure of the olfactory threshold and a comparison of the midpoints of different acids gave an estimate of their relative detectability. As Cheesman & Mayne (1953) have pointed out in a similar context, little reliance can be placed on the fiducial limits de-

rived by application of probit analysis to data such as the present and these values have therefore been excluded in assessing the results.

3. Estimation of Acid Vapour Concentration

The median threshold values initially determined in this way are expressed as concentrations in solution and the concentrations of odorous vapours above the solutions have to be estimated from this data. The procedure is complicated in the fatty acids since, being weak electrolytes, they dissociate to varying extents in solution and the vapour pressure of the odorant above a solution derives from the undissociated acid. In the case of members which have limited solubility in water a relatively good approximation can be obtained from the following relation:

$$Ps = Pv \times \frac{C_1}{C_2}$$

where Ps=the vapour pressure of the acid in dilute solution,

Pv=the saturated vapour pressure of the pure acid and can be calculated for the temperature of the experiment from tables given by Driesbach (1952).

C_1=the concentration of the undissociated acid at median threshold

C_2=the concentration of the undissociated acid in saturated solution.

C_1 and C_2 can be calculated from the equation $K = \frac{(Cx)^2}{C - Cx}$ where K is the dissociation constant of the acid, Cx is either C_1 or C_2, and C is the total concentration of the acid in solution at median threshold.

In the case of the acids which are completely miscible with water the best estimate is derived from the relation:

$Ps = Pv \times M$, where M is the mole fraction of the acid in solution (i.e. the proportion of the total number of molecules in solution which are acid molecules), and Ps and Pv are as above.

Results

It is apparent from the results given in Table I that there are marked individual differences in the responses of the two dogs to these acids. One of these differences is emphasized in Fig. 4 where the median threshold values are plotted against the number of carbon atoms in the molecule. Thus with the exception of caproic, Dick detected the acids at progressively decreasing concentrations as the series is ascended. In contrast, the data for Venus show a striking discontinuity: the first four and the remaining four acids form two groups within each of which stimulating efficiency increases as chain length is expanded. These groups differ in two main respects: (1) response increments between successive members are 1·54—1·82 log units of molar concentration in the first group and only 0·739—0·801 log units in the second group; (2) if, in Fig. 4, a regression line be calculated for the first four members, acids of the second group are found to be displaced upwards by about 2 log units from an extrapolation of this line, instead of tending to lie on it as in the equivalent situation for Dick.

The data for Venus can thus be represented by two regression lines which describe the tendency of members of the two groups to increase in detectability as the series is ascended. When chain length is expressed on a logarithmic scale the lines are parallel, but if an arithmetic scale is used they diverge. For this reason a logarithmic scale of chain length has been used in Fig. 4. However, although the data for Dick may be determined largely by some factor which alters progressively and logarithmically as the series is ascended, it is clear that some other effect is also operating.
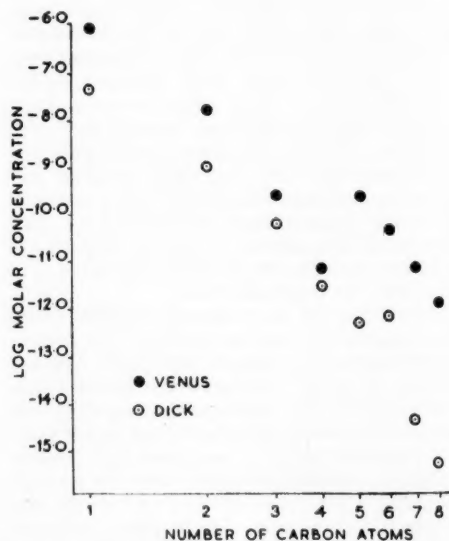

Fig. 4. Detectability of the n-aliphatic acids by two dogs. The log. molar concentration of the acid vapour at median threshold is plotted against chain length.

Table I. Detectability of the n-aliphatic acids by Two Dogs. The column headed "Number of presentations" refers to the number of times a dog was required to make a choice.

Dog	Acid	Number of presentations	Slope of probit regression line	Molar Concentration of solution at median threshold	Molar Concentration of vapour at median threshold	Thermodynamic activity at median threshold
Venus	Formic	1728	0·903	—1·515	—6·087	—3·258
	Acetic	3648	0·787	—2·541	—7·743	—4·285
	Proprionic	1728	0·860	—3·580	—9·565	—5·324
	Butyric	2688	0·513	—4·632	—11·109	—6·376
	Valeric	3648	0·688	—3·265	—9·523	—4·297
	Caproic	6720	0·935	—3·568	—10·272	—4·566
	Heptylic	2688	0·782	—3·863	—11·073	—4·845
	Caprylic	2688	1·097	—4·151	—11·812	—5·133
	Iso-Butyric	1344	0·957	—4·916	—11·199	—6·660
Dick	Formic	3840	1·755	—2·735	—7·383	—4·478
	Acetic	2496	0·661	—3·838	—8·993	—5·582
	Proprionic	5376	0·715	—4·404	—10·216	—6·146
	Butyric	5376	0·565	—5·082	—11·462	—6·826
	Valeric	4032	0·517	—5·294	—12·218	—6·909
	Caproic	2688	0·474	—4·875	—12·091	—6·175
	Heptylic	2688	0·933	—6·122	—14·318	—8·248
	Caprylic	1344	0·549	—6·298	—15·217	—8·609

The results given in Table 1 show also that at median threshold iso-butyric acid is detected at lower concentrations than its normal isomer.

Slopes of Probit Regression Lines

It was found that in many cases the slopes of the stimulus-response curves for replications of a given acid showed marked divergences, and in an attempt to reduce the influence of this source of variability, additional replications were made where they seemed advisable. This largely accounts for the discrepancies in the number of presentations given for each acid in Table I. However, since no reliable estimate of fiducial limits can be made (see "Methods"), it is not clear to what extent these precautions have been successful. At any rate there is much variation in the slopes of the probit regression lines for each acid (see Table I), and they do not decrease logarithmically in value as do the equivalent data in the previous studies in this series (Moulton & Eayrs, 1960; Moulton, 1960). Nevertheless their distribution does not seem to be entirely random: there is a tendency for the lowest values to occur towards the centre of the range of acids studied, and since this trend occurs in both dogs, some significance may be attached to it. Its main implications are that the pattern of detectability is a function of concentration and may, at certain success levels, reflect the same oscillation as is seen in the values for slope. The discontinuity present in the median thresholds for Venus may therefore also occur at some level in the data for Dick. The underlying features in the responses of the two dogs may thus show closer resemblances than the median threshold values suggest.

In fact, if the concentrations of acids corres-

ponding to the 90 per cent. success level are estimated by substituting this value in the probit regression equations, and plotted against chain length, as has been done in Fig. 5, the similarity of the two patterns is at once striking. The discontinuity which is already apparent in the

corrections for day-to-day fluctuations in performance were not applied to the data, this is a reasonable interpretation. However, it was shown in a previous study (Moulton, 1960) that the stimulus-response curve of each member of a series of esters of acetic acid above the second
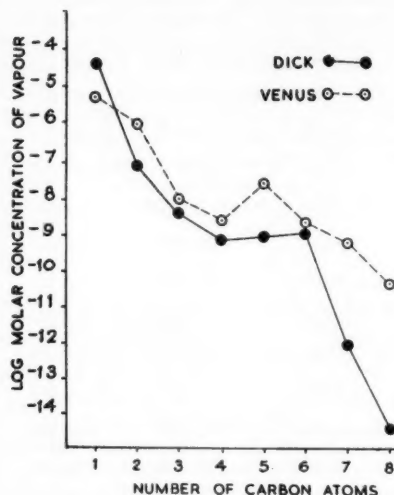

Fig. 5. Relation between detectability of fatty acids by two dogs and carbon chain length. The values of molar concentrations of vapour are those corresponding to a 90 per cent. success score.

median threshold values for Venus, persists, but an oscillation now appears in the success scores for Dick and in keeping with this, scores for members containing 2-6 carbon atoms occupy a concentration range of two log units instead of three. However, the sharp increase in detectability which occurs as the series is ascended through the higher members, is in marked contrast to this trend, and suggests that a different set of factors may be influencing the stimulating efficiency of these members.

Stimulus-response Curves

If the stimulus-response curve for each replication of an acid is plotted before applying the probit transformation it is found that in almost every case, one, two or occasionally three reversals of slope are present. As can be seen in Fig. 6, which is an example of such a curve, these reversals may be so small as to suggest that they are the result of experimental error and since
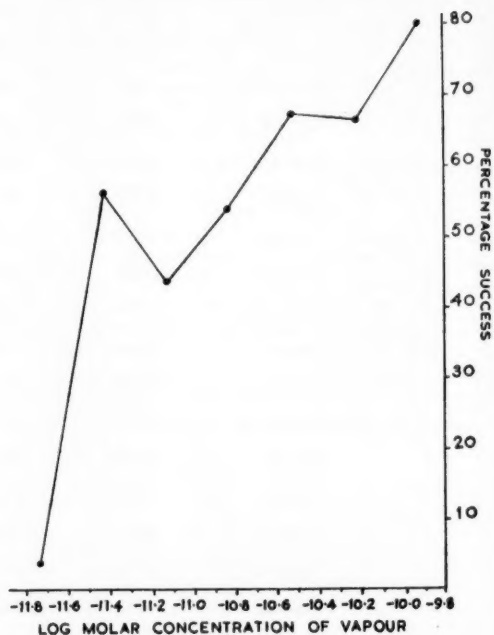

Fig. 6. Relation between vapour concentration of butyric acid and detectability by a dog (Dick). The curve shows one marked reversal with a trough at —11·13 log units of concentration and a peak at —11·43 log units, and one minor reversal with a trough at —10·23 log units and a peak at —10·53 log units.

contains one reversal whose position alters in a predictable way as the series is ascended. In the case of amyl acetate Mozell (1958) has noted a similar reversal in a curve derived from recordings of the electrical activity in the olfactory bulb of the rabbit. The present anomalies therefore merit consideration.

Reference to Fig. 6 will show that the reversals can be expressed by either the concentration or the response levels at which their troughs appear. When expressed as response levels and plotted against chain length, no clear trend, such as was present in a previous study, using the rat (Moul-

ton, 1960) is discernible, although in some acids at least, the troughs occur at the same points on the curve in successive replications.

However, if the concentrations at which the troughs appear are expressed as average values for each acid and plotted against chain length, as in Fig. 7, it can be seen that they tend to occur at logarithmically decreasing concentrations as the series is ascended. The striking feature of this result is that the data for Venus follow a pattern which is closely similar to that for Dick and do
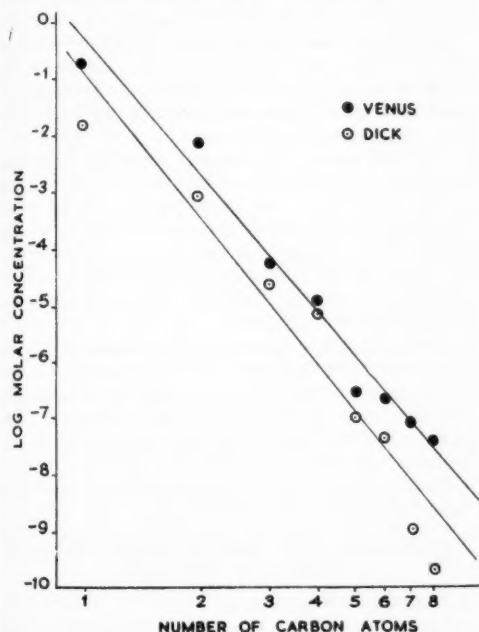

Fig. 7. Average vapour concentrations at which the troughs of reversals (see text) appear on the stimulus response curves for the detectability of the fatty acids. Values are given for two dogs and are plotted against chain length.

not show the displacement effect characteristic of median threshold concentrations (Fig. 4), or of the concentration range used. This suggests that some reversals at least, may be associated with olfactory events rather than with randomly determined experimental errors. It can therefore be argued, as has been done in relation to the aliphatic acetates (Moulton, 1960), that these reversals are due to the presence of two or more olfactory receptor types, certain of which attain

maximum response at lower concentrations than do the remainder. Such an explanation implies that the stimulus-response curve for olfaction, in certain compounds at least, may represent two or more overlapping elements as is seen in the critical flicker fusion curve for vision.

The asymptotes of the curves are reached in about 1-3·5 log units of molar concentration and in many cases there is a decline in success scores as concentrations are increased beyond this point.

Discussion

Effectiveness of Technique

In the preliminary account of this work (Ashton, Eayrs & Moulton, 1957) reasons were given for assuming that the responses measured were elicited solely by the acid vapour. The failure of the dogs to obtain scores above chance when concentrations were decreased below a certain level supports this view and it is clear that the method effectively eliminates non-olfactory cues. It is possible that the value of the technique might be improved by eliminating the need for a handler. However, as Becker, Markee & King (1957) have shown, there are considerable difficulties involved in training dogs to achieve a consistent performance in the absence of a handler and long periods of re-training are necessary.

Olfactory Acuity in Dog and Man

Adrian (1953) has suggested that, in spite of its superior sense of smell, the dog's absolute olfactory sensitivity may be little greater than man's. He explains this by assuming that the sensitivity of individual receptors is similar in both species but that a large surface covered with receptors can give more information than a small one. This could provide the basis for a fine analysis of odour mixtures which might account largely for the differences in olfactory performance between the species. But such a view is hardly consistent with Neuhaus's (1953) claim that olfactory acuity in the dog is one million to one thousand million times greater than in man. Indeed, this implies an enormous species difference in the sensitivity of individual receptors. It is therefore interesting to see how the thresholds obtained in the present study compare with the equivalent data for man. Before this is done, however, the complexity of factors which obscure such a comparison should be recognized. These include differences in method, in age, sex and motivation of subjects, in the classes of odours which have biological significance for

the two species, in the criterion of response chosen, in the extent to which the olfactory organ is adapted to background odours, in the amount of training given, and in the wide variation in human olfactory thresholds reported for the same substances. Further, since the area of the nasal mucus sheet is much larger in the dog than in man, its ability to absorb odorous molecules may also be greater (cf. De Vries, 1956). Of the molecules entering the nasal cavity the proportion which eventually reach the receptor surface may therefore differ in the two species.

Because of these factors, only a very crude estimate of relative olfactory sensitivities can be made. However, when this is done it suggests that, for butyric acid for example, the dogs used in the present study have an olfactory sensitivity ranging up to 100 times greater than the lowest thresholds reported for this compound in man (Passy, 1892; Backman, 1917; Skramlik, 1948). This estimate is consistent with subjective observations made during trials.

Now an even greater discrepancy between olfactory sensitivities of dog and man might still be accounted for by the suggestion that the larger area of olfactory receptors which occurs in the dog, is more likely to contain receptors of unusually low threshold than the smaller area present in man (Adrian, 1953). Further, it is possible that summation of impulses is more effective in the large olfactory bulbs of the dog than is the case in man. Both of these effects could result in a lower threshold in the dog as determined behaviourally but in neither case do they necessarily imply a difference in the average sensitivity of the olfactory receptors between the two species. However, the general problem of species differences in olfactory receptor sensitivity is one which requires further studies incorporating electrophysiological techniques, and the essential point is that the present threshold values for the dog are not impressive when compared with corresponding values for man. The implication is that the superior olfactory powers of the dog may stem less from an extreme absolute olfactory sensitivity than from its ability to discriminate between different odours—a capacity which, by all available evidence, is highly developed in this species (Kalisher, 1909; Heitzenroeder, 1913; Seffrin, 1915; Bierens de Haan,1926; Buytendijk, 1921; Löhner, 1926; Kalmus, 1955; Neuhaus, 1956a, 1956b).

Relative Detectability of the n-aliphatic Acids by the Dog

In a number of homologous series of odorants the relative olfactory stimulating efficiency of their members has for long been the subject of conflicting claims. In particular, thresholds for the formic acid series reveal an especially extensive range of response patterns. Nevertheless, until recently, it has generally been found that detectability increases as the series is ascended to the 3rd, 4th and 5th member, and then declines. Such studies include those of Passy (1893), Backman (1917), Hesse (1926), Skramlik (1948) on man, Neuhaus (1953) on the dog, Gruch (1957) on the rat, and Ottoson (1958a), who recorded the slow potentials evoked in the olfactory epithelium of the frog.* Of these workers Passy, Hesse, Skramlik and Ottoson found a further phase of increasing stimulating efficiency, and this is essentially the pattern shown in the data for Venus (Fig. 4).

In 1957, however, Hughes reported that the olfactory stimulating efficiency of fatty acids toward blowflies and tsetseflies increases progressively as the series is ascended and it is this trend which appears in the data for Dick (Fig. 4). Thus in this series, as in others whose odour intensity has been studied, there is a sharp dichotomy of findings.

In considering the possible causes of these divergencies it is convenient to begin by examining two explanations, the first of which has already been advanced to explain the break in the data for Venus, and the second of which involves the influence of factors peculiar to experiments with dogs. The remaining possibilities are of more general application.

Possible Causes of Anomalies in the Pattern of Response to fatty Acids

1 Trigeminal Response

Allen (1937) found that dogs with bilaterally ablated olfactory bulbs could still detect the vapour of acetic and butyric acid whilst dogs which had, in addition, sectioned trigeminal nerves were unable to do so. It thus seemed possible to account for the break in the data for Venus by assuming that whilst the free endings of the trigeminal nerve in the nasal mucosa are irritated by the first four acids of the series, remaining members have little or no such effect (Ashton, Eayrs & Moulton, 1957). Allen, however, apparently used concentrated solutions of the acids, and all available evidence suggests that most irritants only irritate at high concen-

*Data for this series are also given by Mitsumoto (1926), and Watson (1922) but values for members above butyric are not included.

trations (Parker & Stabler, 1913; Katz & Talbert, 1930). For example, Schiff (1859) claims that pups with severed olfactory nerves would respond to acetic acid only in very concentrated solution. In the present study all concentrations used were extremely dilute and it is therefore unlikely that the pattern of response found was influenced to any significant extent by trigeminal sensitivity.

2 Effect of Adaptation

Olfactory thresholds for a given odour are higher if a period of adaptation to this odour or related odours precedes measurement (Zwaardemaker, 1925; Cheesman & Mayne, 1953). In the present study a rise of threshold might therefore occur if odours similar to those being tested were present in the experimental environment. Although there is no reason to suppose that the precautions taken to ensure circulation of fresh air in the experimental hut were not sufficient to eliminate such effects, it is nevertheless a possibility that requires consideration. The most likely sources of such contamination would appear to be human and canine body secretions. However, with the possible exception of heptylic acid, human sweat contains all members of this series with 1-8 carbon atoms (Nitta & Ikai, 1953; Kuno, 1956) and is therefore unlikely to account for the effect.

On the other hand, the volatile fatty acids in the hair grease of the dog consist almost entirely of valeric acids (Brower & Nijcamp, 1953). Now, Haycraft (1889) claims that there are qualitative differences between the first four (formic-butyric) and the next four (valeric-caproic) acids, and this is consistent with Uchida's (1955) observation that the behaviour of dogs in a tracking experiment differs markedly towards the two groups. If, in fact, valeric acid has especially close qualitative affinities with caproic, heptylic and caprylic acids it is possible that its presence in the experimental environment would lower thresholds not only for itself but also for all odorants of the second group.

However, it is difficult to believe that the data for Dick would show no evidence of such an effect, were it operating, and for this reason it seems unlikely that adaptation to such contaminants had any influence on the present data.

3 Impurities in the Test Odorants

Backman (1917) has suggested that anomalies in the detectability pattern of the fatty acids may be due to the presence of impurities. Such an explanation, however, can hardly explain divergences in the present data since the acids used in experiments with both dogs were taken from the same set of bottles.

4 Physico-chemical Properties of the Fatty Acids

The only property of the fatty acids which shows a break in its relation to chain length similar to that appearing in the thresholds for Venus (Fig. 4), is water solubility. Thus the first four acids are miscible in water in all proportions whilst valeric and higher acids show progressively declining solubility as the series is ascended. For example, valeric acid is soluble only to the extent of 3·3 parts in 100 parts of water. The interpretation which this immediately suggests is that for members above butyric acid, water solubility is a limiting factor. This can also explain why, as concentrations are increased, the response pattern for Dick changes from a linear to an oscillating pattern. In fact such a view has already been developed in relation to a similar transformation in the detectability pattern of alcohols (Moulton & Eayrs, 1959), and since it assumes the presence of some aqueous phase (e.g. the olfactory mucus) associated with the olfactory receptors, it could account for differences between the pattern of response for Dick and Venus. Thus the quantity of this phase (e.g. the thickness or composition of the mucus sheet) which is available, may vary between individuals. Consequently at a given concentration of the odorant the volume of this phase in one subject might be so small as to limit stimulating efficiency, whilst in another subject its volume would be sufficiently large to avoid this.

There is, however, a further factor associated with water solubility which may complicate response to these acids. Unlike other series whose odour intensity has been studied frequently, the fatty acids are weak electrolytes. It is thus possible that acid vapours will dissolve in the olfactory mucus sheet and ionize to an extent determined by their dissociation constants and their water solubility. Now since olfactory receptors are highly sensitive to changes in their ionic environment (Adrian, 1949; Ottoson, 1958b) this effect could augment stimulating efficiency, and the smaller the limiting influence of an aqueous phase the greater the enhancement would be. However, if this factor operates, its influence is probably small since formic acid, with a dissociation constant about ten times that of the remaining acids, is not more effective than acetic acid, as is the case in other biological

systems where it is concluded that the degree of dissociation of these acids plays a prominent role in determining their effectiveness (e.g. Stiles & Rees, 1935; Yonge, 1936). On the other hand the anomalous slope for formic acid in the data for Dick may be related to such an effect.

But a theory based on the water solubilities of these acids can account for but a single oscillation in response, and olfaction in the fatty acids appears to be unique in that in many studies a second phase of increasing odour intensity has been reported. Thus in the data for Venus a decay in response occurs between only two members of the series (butyric and valeric). Thereafter effectiveness again increases as the series is ascended although thresholds of higher members occupy a much smaller range of concentrations than do lower members. This suggests that some property of the molecule whose effectiveness increases as chain length is expanded is able to compensate to a certain extent for limited water solubility. Possibly a two phase system such as that postulated by Chadwick & Dethier (1949) to account for the limiting mechanism in tarsal chemoreception in the blowfly may also explain certain features of olfactory response to fatty acids. According to this view smaller molecules gain access to the receptors in part through an aqueous phase while the larger molecules penetrate through or accumulate in a lipoid phase. But further studies are clearly needed.

These concentrations suggest that the final pattern of olfactory stimulating efficiency in an homologous series such as the fatty acids, may reflect the influence of several properties of the molecule, some of which are antagonistic, others complementary in their effects. The particular pattern which emerges may be determined in part by individual and species differences. Nevertheless there are grounds for supposing that a linear rather than an oscillating pattern of response is more likely to be significant in understanding relations between olfactory stimulating efficiency and the physico-chemical properties of these acids. Thus a linear relation between activity and chain length is characteristic of the action of aliphatic compounds on a wide range of biological systems including the toxic action of fatty acids towards potato root tubers (Stiles & Rees, 1937), their efficiency in stimulating the killifish, *Fundulus heteroclitus* (Allison & Cole, 1934) and the tarsal chemoreceptors of the blowfly, *Phormia regina* (Chadwick & Dethier, 1947). It is also the pattern shown in the ol-factory responses of rats to alcohols and acetates (Moulton & Eayrs, 1959; Moulton, 1959) and by many of the physical properties of homologous series.

Analysis in Terms of Thermodynamic Activities

It has been found that, in a number of homologous series, members of intermediate chain length are about equally stimulating when expressed as thermodynamic activities[*] (Dethier, 1954a; Dethier & Yost, 1952; Ottoson, 1958a; Moulton & Eayrs, 1959; Moulton, 1959). As Dethier (1954b) has pointed out, such a relation suggests that the phenomenon observed may involve the establishment of an equilibrium and it can therefore be argued that it represents a physical rather than a chemical process. When such an analysis is applied to the present data (Table I), it can be seen that the relation again tends to hold especially in the case of Venus. In the data for Dick median threshold values for members of 2-8 carbon atoms, when expressed as thermodynamic activities, occupy a range of log units which is about half of that when expressed as molar units. However, there is a tendency for the acids to be detected at progressively decreasing activities as the series is ascended and in this respect the pattern for Dick is closely similar to the equivalent pattern for alcohols in rats (Moulton & Eayrs, 1959), even including the inflecton at the sixth member. This failure of short-chain members to stimulate at equal thermodynamic activities is the one consistent feature in all the available evidence concerning olfaction in homologous series (cf. Moulton & Eayrs, 1959), but whether it is related to the poor oil solubility of these members as Ottoson (1958a) has suggested or to some other factor is not clear. It is evident, however, that in many features the pattern of detectability at median threshold is similar, whether expressed in molar or activity units. In particular, individual differences, which are a prominent feature of olfactory data (cf. Jones, 1957), remain.

Conclusions

These results show that individual differences may be as important in determining variations

[*]The thermodynamic activity of a compound is defined as its partial free molar energy referred to a standard state and is numerically equivalent to the relative saturation of the vapour of the compound. For a fuller discussion of the rationale behind the application of analysis in terms of thermodynamic activities see Ferguson (1939, 1951), Ferguson & Pirie (1948), Brink & Posternack (1948), and Dethier (1954b).

in the relative detectability of members of an homologous series as species, technical or other differences. They illustrate the need for using a large number of subjects in experiments of this kind if broad conclusions are to be derived. Because of this factor the rat is preferable to the dog as a subject in studies of olfaction in homologous series.

Summary

The ability of two dogs to detect the first eight members of the formic acid series in the vapour phase has been investigated and a behavioural technique for determining the dog's olfactory thresholds is described. In one dog, detectability tended to increase logarithmically as the series is ascended, but in the other this relation is discontinuous: the fifth member is less easily detected than the fourth and higher acids are displaced proportionately in their effects. It is suggested that this anomaly is determined by a corresponding break in the water solubilities of these acids. When expressed as thermodynamic activities the first four acids are detected at decreasing activities as the series is ascended whilst members of intermediate chain length are about equally stimulating.

In both dogs reversals are present in the stimulus-response curves which tend to occur at logarithmically decreasing concentrations as the series is ascended. There is no break in this relation.

It is concluded that the threshold data presented are not inconsistent with Adrian's suggestion that the sensitivity of individual olfactory receptors is similar in man and dog.

Acknowledgments

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STUDIES IN OLFACTORY ACUITY. 5. THE COMPARATIVE OLFACTORY SENSITIVITY OF PIGMENTED AND ALBINO RATS

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Introduction

Although albinos, of certain species at least, appear to have inferior visual and auditory powers (see for example Lashley, 1930; Wolff, 1942; Hermann, 1958) the evidence that they possess poor olfactory sensitivity is less convincing. The suggestion that albinism and anosmia are associated (e.g. Ogle, 1870; Moncrieff, 1951; Allison, 1953; Young, 1957) is largely based on anecdotal material. Darwin (1868) for instance quoted examples to illustrate the immunity of dark skinned animals to vegetable poisons, stating that, in certain parts of Florida, all the pigs except the black ones suffer from eating the roots of *Lachnanthes tinctoria*, whilst in Sicily only white sheep are poisoned by eating *Hypericum crispum*. Such examples prove little about olfactory sensitivity, since, as Darwin himself implied, all the animals may eat the poisonous plants but only the black ones survive. And even if sensory discrimination were involved it could equally depend on non-olfactory cues less readily detectable by albinos. It is nevertheless claimed to be common knowledge that some albinos are congenitally hyposmatic or even anosmatic (McCord & Witheridge, 1949).

The possibility of an association between albinism and an inferior sense of smell is clearly significant for theories which allot a role in the olfactory process to the pale-yellow to dark-brown pigment of the mammalian olfactory organ (Heyninx, 1919; Gerebtzoff & Philippot, 1957; Wright, 1957). There is some experimental support for these suggestions since Adrian (1956) has observed, in the course of studies of the electrical activity of the olfactory bulb, that a deeply pigmented organ is more sensitive to odours than one with little colour. It has been claimed (e.g. Young, 1957) that albinos lack this pigment and although this contention derives little support from comparisons of pigment densities in black and albino rats, whether in terms of gross appearance or of the results of

fractionating extracts of olfactory epithelium by paper chromatography (Moulton, 1958), it is nevertheless possible that some element of the olfactory pigment complex which might influence olfactory sensitivity is lacking in albinos. In this context, evidence of a poor sense of smell in the albino rat would be of interest and in fact Keeler (1942) has obtained data which could be taken as providing such evidence. In this study he crossed albino rats carrying a recessive gene for black coat colour with wild grey Norway rats (*Rattus norvegicus, Berkenhout*), and used 125 of the F2 and backcross segregates to determine whether behavioural differences existed between these black, grey and albino rats. In one of his tests a piece of garlic was held 2 mm. from their noses: 71 per cent. of the albinos backed away after sniffing the garlic for an average of 9·9 seconds while 66 per cent. of the pigmented rats backed away after an average of 4·9 seconds. When this test was repeated using 10 Hatai black hooded rats and albinos, the black rats backed away after 7 seconds whilst the albinos never backed away. On the basis of these experiments Keeler postulated that the gene for albinism has a profound dulling effect on olfactory sensitivity. More recently, Barnett & Spencer (1953) suggested that some support for this hypothesis lay in their finding that the wild Norway rat will avoid butyric acid and aniseed, substances earlier shown to have negative properties for albino rats (Scott & Quint, 1942).

However, these results are open to other interpretations. In high concentrations butyric acid and allyl sulphide (which is emitted by garlic) appear to be trigeminal irritants (Katz & Talbert, 1930; Allen, 1935) and it is possible that the rats were responding not to an olfactory but to an irritating component of the test vapour. Behavioural differences between pigmented and albino rats might also account for the discrepancies. It is true that a superior sense of smell might be expected in the wild Norway rat since its olfactory bulbs are heavier and larger than those of the albino and contain a larger number of granular cells (Holt, 1917; Smith, 1928). But

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the domestication of the albino rat rather than the effect of the albino gene could account for these discrepancies. Moreover, evidence which directly conflicts with Keeler's hypothesis is Gruch's (1957) failure to find any significant difference between the olfactory powers of albino and pigmented rats, although the sensitivity of the techniques which he used has been questioned (Eayrs & Moulton, 1960).

In view of the extensive use of the albino rat in behavioural experiments, and its suitability as a subject in the study of olfactory sensitivity (Pfaffman, Goff & Bare, 1958; Eayrs & Moulton, 1960), this is a question of some interest and it was therefore the aim of the present study to provide data on the relative olfactory ability of pigmented and albino individuals.

Materials and Methods

Rats

The rats were obtained as follows: A strain of tame black Norway rats (*Rattus norvegicus*, Berkenhout) which had been bred under laboratory conditions for many generations, and albino rats of Birmingham University stock were crossed to give pigmented progeny. These F1 hybrids were interbred to give litters, some of which contained both pigmented and albino males and only such littermates were used in this study. Females were excluded in view of claims that olfactory sensitivity is influenced by the ovarian cycle (Elsberg, Brewer & Levy, 1935; Le Magnen, 1950; Schneider & Wolf, 1955). The animals were raised under as nearly identical conditions as possible, and weaned when 21 days old. Initially 18 black and 12 albino rats were used, the experiment being subsequently repeated using 24 black and 24 albino rats.

Odorants

The n-aliphatic alcohols used as test compounds were distilled before use and diluted in logarithmically descending concentrations with propylene glycol. All compounds were obtained from British Drug Houses Limited.

Apparatus

The circular choice apparatus used has been fully described by Eayrs & Moulton (1960), and only a brief account will be given here. It consists essentially of two concentric sheet metal cylinders, the outer of which is free to rotate about the inner. Two drinking bottles are attached at opposite ends of the outer cylinder so that their spouts project into the central arena,

under a gap between the inner cylinder and the floor of the apparatus. Each spout bears a container into which a disposable nylon capsule can be fitted and into which in turn is placed a cotton wool pellet. The pellet associated with one bottle is impregnated with a solution of the test odorant whilst the remaining pellet is impregnated with the control solution of propylene glycol. An electric shocking device is connected to the bottle carrying the odorant and to the copper grid floor of the apparatus in such a way that rats drinking from this bottle receive a shock whilst the control bottle is shock-free.

Conduct of Trials

The method of conducting and scoring trials was, with minor modifications which are noted below, essentially that described fully by Eayrs & Moulton (1960).

Rats were habituated to the experimental situation by placing them in the apparatus for two nights with the outer cylinder rotating at about one revolution in 12 hours, and the shocking system attached to the spout carrying the odorant. They were deprived of water for 24 hours and tested on a series of dilutions of the odorant (the appropriate range of dilutions was determined on the basis of previous experience with newly weaned rats), presented according to a 7 × 7 balanced incomplete block design. This allowed seven concentrations to be presented in groups of four daily for seven days in such a way that each concentration was tested on four different days. On any day the order of presenting the dilutions was randomized and owing to the number of rats used the intervals between trials for any one rat were long. There was thus little opportunity for adaptation to the test odorant to occur. The rats were tested eight times daily: twice on each of four dilutions. They were introduced into the apparatus manually and the positions of the drinking spouts were set at random by rotating the outer cylinder between individual trials.

In the first experiment threshold values for n-hexyl alcohol were determined twice: initially when the rats were on average about 35 days old and experimentally naïve at the start of the experiments, and finally when the average age was about 160 days. In the interval between these occasions the rats were trained and tested on other odorants (ethanol and butanol). In the second experiment rats were tested on n-hexyl alcohol when they were on average about 40 days old.

During the first series of observations the rat's score alone was noted, but during the second the number of occasions on which each rat failed to make a choice within 100 seconds of being placed within the apparatus was recorded in addition.

Statistical Procedures

In assessing the significance of differences in olfactory performance between the two groups of rat, the "t" test was applied to the crude aggregate daily scores for each rat, but in determining median thresholds, corrections were applied for day-to-day fluctuations in performance and the results subjected to probit analysis. The resulting values were converted to molar concentrations of vapour by the method described by Moulton & Eayrs (1960).

Results

The results (Table I) show that, although the pigmented rats performed better than the albinos during the trials conducted at 35 days old, the position was reversed at 160 days. There

Table I. **Detectability of n-hexyl alcohol by 18 Black and 12 Albino Rats at Different Stages of Training.** Thresholds are expressed as molar concentration of vapour at which rats obtained 50 per cent. success scores. Each value is derived from a block of 56 trials in which 7 concentrations were tested.

Approximate average age of rats in days	Log molar concentration of vapour at median threshold	
	Black rats	Albino rats
35	—8·093	—7·507
160	—8·308	—8·682

would thus appear to be no direct and consistent relationship between pigmentation and olfactory sensibility. The reliability of fiducial limits for thresholds derived from probit analysis has been questioned, however, and the significance of these trends was therefore assessed by examining the crude data—a procedure which also served to emphasize the element of inconsistency in the results and to suggest a possible explanation. Thus it appears that during the first 24 trials of the period in which the rats were on the average 35 days of age the scores of the black group were significantly higher than those of the albinos (P<0·001). However, the performance of the albinos improved rapidly so that it exceeded that of the black rats by progressively

higher amounts on ethanol, butanol and hexanol, the values in terms of the average number of correct responses being 0·17, 0·51 and 2·15 respectively. In no case was the difference statistically significant. Furthermore, although in the course of the initial period of training rats of both types showed exploratory behaviour, grooming, and 'freezing', and made repeated approaches to and withdrawals from the drinking spouts before making a choice, it seemed that during the first series of trials, the black rats behaved more cautiously than did the albinos and that they required longer to make a choice. This observation was confirmed by the second series of trials when the number of occasions on which rats failed to make a choice within the first 100 seconds of the test was noted. It was found that the black rats reached this time limit significantly more frequently during the first eight trials than did albinos (38:22, P<0·001).

Discussion

The essential finding of this experiment is its failure to show any difference between the olfactory sensitivity of fully trained pigmented and albino rats. This confirms the similar conclusion of Gruch (1957) and is consistent with the inability of Foster (1950) to find in man any significant difference in the olfactory sensitivity of near-albinos and negroes. It also accords with the failure of Beidler & Tucker (1960) to detect, in pigmented and albino rabbits, any difference in the electrical activity of primary olfactory nerve fibres in response to odour. Thus, in the male rat at least, there is clearly no association between albinism and anosmia, and Keeler's (1942) hypothesis that the albino gene has a profound dulling effect on the sense of smell is not supported at least as far as the odorants used in the present experiment are concerned.

Nevertheless, in the early stages of training, the albino rats of the present study made scores which were significantly lower than those of the black rats. The difference diminished rapidly until it was no longer significant. It is perhaps possible that this phenomenon reflected changes in the relative sensitiveness of the olfactory systems of the two groups of rats, but a more probable explanation is that behavioural differences were involved. Several traits in the rat, including emotionality and exploratory behaviour, appear to be at least partially determined by genetic factors (e.g. Hill, 1941; Farris & Yaekel, 1945; Carr & Williams, 1957), and in particular, there

is some evidence that characteristics of this kind may be associated with the genes determining coat colour (Keeler, 1942; Keeler & King, 1942). It is thus reasonable to suppose that in the present study the two groups of rats differed in some trait, such as emotionality, with the result that the pigmented rats displayed a more pronounced approach-avoidance conflict in the odour choice situation. It is also consistent with Barnett's (1958) observation that, when faced with a new object, hooded rats are more wary than albinos, a difference which he suggests may be related to the superior visual acuity of the pigmented animal.

Now it is not difficult to see how such factors might influence olfactory response. Thus the more hesitant the rat in the odour choice situation, the more probable it will be that the concentration of the test compound in the nasal cavity will reach threshold levels, since this behaviour is usually associated with vigorous sniffing. The present results could therefore be explained by assuming that the black rats were initially more hesitant than were the albinos but that with further training this tendency was rapidly overcome. In any case it seems clear that when the responses of pigmented and albino rats to compounds in the vapour phase is being compared, as in Keeler's (1942) study, the results must be interpreted with considerable caution and it cannot be readily assumed that any differences which emerge reflect differences in the olfactory capacities of the two groups of rats.

Finally it should be noted that the present conclusions in no way alter the possibility that pigment plays either a primary or a secondary role in the olfactory process. This remains a difficult problem on which much research is needed.

Summary

1. Literature concerning possible relations between pigment and olfactory acuity is briefly reviewed.

2. The detectability of n-hexyl alcohol by 18 black and 12 albino male rats (F_2 progeny) of a cross between black and albino strains) has been investigated using an odour choice apparatus.

3. In the first 24 trials of training black rats made significantly higher scores than did albinos ($P<0.001$), but in fully trained animals the position was reversed although the difference was not significant.

4. It is suggested that there were inherited behavioural differences between the two groups

of rats which could account for these results.

5. The hypothesis that the albino gene has a profound dulling effect on olfactory acuity (Keeler, 1942), is not supported by these findings.

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THE CLASSIFICATION OF SOCIAL STIMULI: "SOCIAL" AND "NON-SOCIAL" DISTRACTION IN THE ALBINO RAT*

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Several forms of social behaviour have been studied in rats (Crawford, 1939; Smith & Ross, 1952). In general, this work supports the position that social behaviour is not qualitatively different from non-social behaviour and is susceptible to the same type of analysis.

Experimental work on the stimulus factors in social behaviour has lagged behind the work on response factors and their determinants. Thus, although many behaviour theorists accept the assumption of continuity between social and non-social stimulation (e.g. Allport, 1924, pp. 147-148; Keller & Schoenfield, 1950, ch. 11; Miller & Dollard, 1941), experimental evidence is lacking. This report summarizes an attempt to arrive at a more precise definition of the rat as social stimulus. A method of investigating social and non-social distraction in rats is described. Evidence is presented which suggests some of the physical dimensions making up the multi-dimensional continua of social stimulation.

Schema

It is well known that a rat's maze performance can be modified by the sudden addition of an extraneous stimulus. In general, any detectable change in a stable level of performance may be defined as a *distraction*, and any identifiable change in the stimulus complex leading to this distraction may be defined as *distracting stimulus*. With these considerations in mind we accepted the following working hypothesis: distracting effects are some monotonically‡ increasing function of the complexity of the distracting stimulus.

Since we were interested in analysing the rat as social stimulus, the stimulus complex, "live rat," was selected as our starting point. Two other, presumably less complex, distracting stimuli were then chosen on the basis of their similarity to the live rat, similarity being defined from the point of view of the experimenters.

If these stimuli were adequately distributed along the assumed continua of complexity, our working hypothesis would suggest the finding of a gradient of distraction. The social stimulus, which is assumed to be the most complex, should produce the most distraction. The other, less complex stimuli should produce decreasing amounts of distraction.

Method

Subjects and Maintenance

The subjects (S) were 20 male albino rats, two months old at the start of the study. On the basis of preliminary work, it was decided to house them in pairs in order to reduce aggression in those animals that were to face social distraction later in the experiment. Each cage contained one S which was to be trained to run to a horizontally striped door and another to a vertically striped door.

The animals were removed from their home cages only for feeding and testing. After all S's were assigned to cages, they were allowed three weeks to adjust to a 24-hour hunger schedule. Each evening they were put into individual feeding cubicles and allowed 15 minutes to ingest 10 grammes of Purina Lab Chow mixed one part crushed pellets to three parts water by weight. They were given a supplement of mixed green vegetables twice a week.

Apparatus

The upper drawing in Fig. 1 shows the wooden four-choice linear maze used during the training periods. The entire unit was painted flat black. The rat was allowed into the entrance area through any of three starting boxes, each of which was operated by a string pulley system,
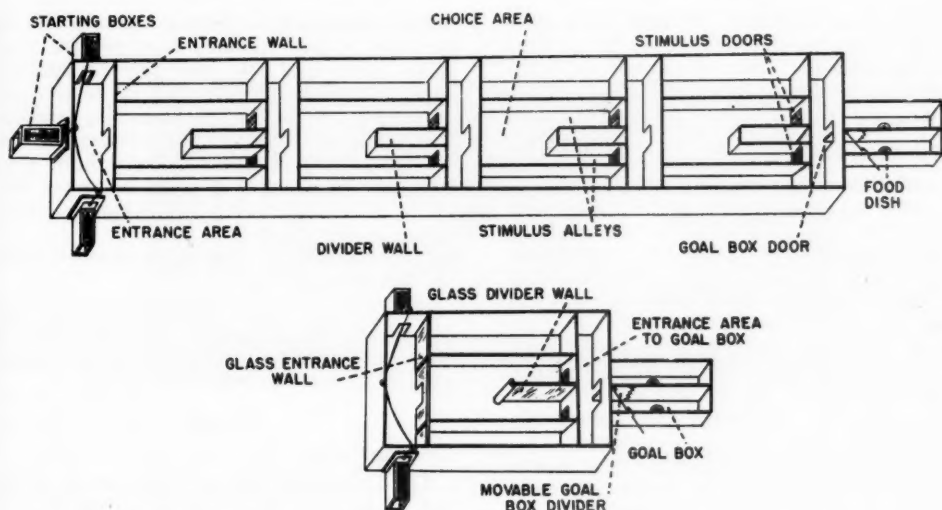
Fig. 1. Discrimination apparatus used during the training and experimental periods.

The distance between the entrance wall and the stimulus doors was 15 inches. The discriminanda consisted of half-inch black and white stripes painted on the stimulus doors, which were three and three-quarter inches square. These doors were interchangeable. The incorrect one was locked from behind, and the correct one was allowed to swing upward to open when the rat pushed it with his nose. The goal box was designed with two compartments to eliminate competition for reward during trials in which S's were run in pairs. The moveable goal box divider could be used to guide the rat into either of the two goal compartments. Two small metal pans were used as food dishes.

The lower drawing in Fig. 1 shows the apparatus used during the experimental periods. It consisted of the goal box and the last section of the linear maze used in the training period. In order to insure good maze visibility during these parts of the study, a glass entrance wall and a glass divider wall were substituted for the wooden ones used during the training period.

Trial Procedure

Duplicate sets of doors were used and alternated from trial to trial to control for possible choice on the basis of visual or cutaneous cues provided by the doors themselves. The S was put into one of the starting boxes and allowed to enter the entrance area. Recording of running time was not begun until the S left the starting box. An error was recorded each time the S entered the incorrect stimulus alley and touched the locked stimulus door with any part of its body. If the S re-entered the incorrect stimulus alley and touched the locked door after returning to the choice area, a second error was recorded. When the subject reached the goal box, it was allowed 30 seconds to consume one gramme of wet mash. The floor of the maze was wiped down with Lysol solution after each trial to mask differential olfactory cues.

Training Period

Ten animals were trained to go to the stimulus door containing horizontal stripes, and ten were trained to go to the vertically striped door. A different, randomized position sequence for the correct doors was used on each trial. The total number of times the correct door was on the left or right side of the maze was made equal to prevent the development of position habits.

All of the S's were given 45 trials on the four-section maze described above. The first ten trials (one per day) were run from the centre-starting box to reduce centrifugal swing effect. The next twelve trials (one per day) were started from the side starting boxes. The position of the starting box was then alternated from trial to trial for each S. As the time scores improved, it became possible to increase the number of trials

given per day. Therefore, the next eight trials were given two per day, and the last fifteen were given three per day. Time in seconds and total errors in traversing the four sections of the maze was recorded.

Experimental Periods

Eight trials were given to facilitate performance in the one-section maze used for the rest of the experiment (see Fig. 1). On the first day, all S's were given four trials with the wooden entrance and divider walls, and on the second day they were given four trials with the glass entrance and divider walls. The last four trials proved especially valuable. Most of the S's became confused by the reflection of the stimulus doors on the glass divider wall and hesitated before going on. Although this temporarily increased time scores, no errors were made.

The rest of the study consisted of three experimental periods, a test period, a distraction period and a retest period, each consisting of twenty-five trials, administered five per day. The starting box position was alternated from trial to trial for each S through all the periods.

Test Period. The incorrect door continued to be locked during this period. These trials established a baseline for stable performance in the absence of distracting stimuli.

Distraction Period. Both doors were unlocked during this period. Thus, errors were no longer punished. The S's were split into four groups during this period.

The control group, G_0, had three S's.* They were run without distracting stimuli, thus continuing the baseline established during the test period.

The stationary-stimulus group, G_1, had four S's. A piece of white cardboard cut in the approximate shape of a rat served as the distracting stimulus. It was mounted on the wall next to the incorrect doorway.

The moving-stimulus group, G_2, had four S's. A toy dog, mounted on wheels, was pulled through the incorrect doorway as soon as S entered the choice alley. The dog was chosen because, from the rear and from the point of view of the experimenter, it presented a reasonable facsimile of a rat moving through a maze.

The fourth group was the live-stimulus group, G_3. It contained four pairs of S's, each pair containing one rat trained with the horizontally

*One of the S's did not learn the maze and had to be discarded at the end of the training period.

striped stimulus door positive and one with the vertically striped stimulus door positive. Cage mates were used for each pair to minimise aggressive behaviour. The first rat to enter the choice alley served as the distracting stimulus. the second rat served as S, providing the data for comparison with the other groups. Since only one following error could occur per trial, an N of four was used in computing average errors for this group.

Plate I shows the distracting stimuli used for the last three groups.

Retest Period. These trials duplicated the conditions of the test period. This was done to measure the stability of any performance changes induced during the distraction period.

Results

Training Period

Fig. 2 shows the time and error scores. By the end of the training period all of the S's had reached a stable level of performance in the four-unit maze.
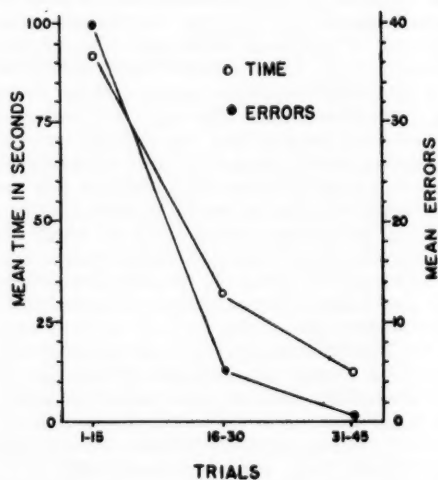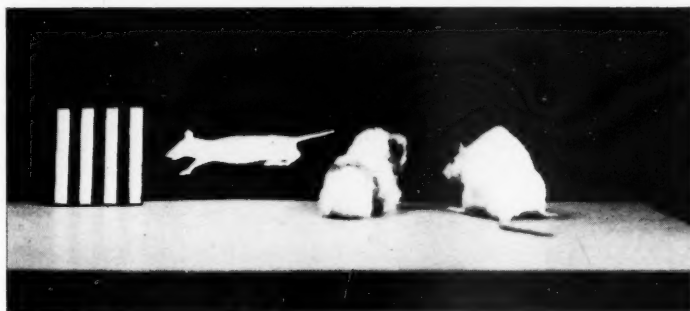

Fig. 2. Time and error scores during the training period.

Experimental Periods

Time Scores. Fig. 3 presents the average time per trial for the three experimental periods. Analysis of variance was applied to the data. The only significant F-ratio was that found between the three periods ($P < \cdot 001$). Thus, the

PLATE I

The stimuli used during the distraction period. From right to left: (*a*) the live rat used in G_3, (*b*) the toy dog used in G_2, (*c*) and the cardboard rat used in G_1. One of the vertically striped stimulus doors is shown on the far left.

PLATE II

A photographic sequence selected to demonstrate typical behaviour shown by G_3 on trials in which the social distracting stimulus produced an error. The action goes from left to right: (1) the S's have just left their respective starting boxes and meet the doorway in the wooden entrance wall. (2) Typical nuzzling behaviour is demonstrated. (3) One of the rats is starting through the doorway into the choice area. On this trial this rat will serve as the social distracting stimulus. (4) The distracting stimulus rat is running down the right stimulus alley and approaching the stimulus door to which it has been trained. The S is following along behind. (5) The distracting stimulus rat is halfway through the correct stimulus door, still closely followed by the S. (6) The distracting stimulus rat is entering the goal box door and the S is going through the stimulus door, thus making an "error". (7) The distracting stimulus rat is eating its reward in the right goal box compartment as the S enters the left compartment (8) Both rats have ingested the food reward and are waiting to be removed for the next trial.

time scores were not influenced by the distracting stimuli. The continued decrease in latency through all periods reflects the increasing stability of performance throughout the rest of the experiment.
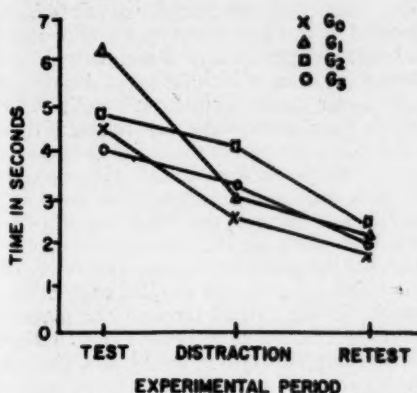

Fig. 3. Average time per trial during the three experimental periods.

Error Scores. Table I contains the raw error scores. Only one error was recorded out of 475 opportunities during the 25 test trials. Thus, the

S's continued their stable level of performance when shifted to the one-unit maze during the test period. It will also be noted that G_0 continued its errorless performance throughout the rest of the experiment.

With a stable baseline established, it was now possible to evaluate any change induced by the distracting stimuli. Table I shows that the number of S's contributing errors during the distraction period increased with the amount of distraction, going from one in G_1, to two in G_2, and including all S's in G_3.

Consistent leader-follower relationships occurred in only one pair of S's in G_3, where Rat 8-H made 11 of 12 errors during the distraction period. Rat 9-H made five errors while leading, in addition to the five errors shown in the table. These were not counted in computing error scores. This animal also had more errors in the retest period than the rest of the S's combined, substantially raising the error scores for G_3 during this period.

Fig. 4 presents the average errors per trial for the three experimental periods. Since only one error occurred in all groups during the test period and none occurred in the control group, G_0, for all periods, these baselines were not included in the analysis.

Jonckeheere's non-parametric test against

Table I. Total Errors for the Experimental Periods.

Group	Rat no.*	Period		
		Test	Distraction	Retest
Control G_0	5—V	0	0	0
	5—H	0	0	0
	6—H	0	0	0
Stationary Stimulus G_1	1—V	0	8	0
	1—H	0	0	0
	2—V	0	0	0
	2—H	0	0	0
Moving Stimulus G_2	3—V	0	11	1
	3—H	1	6	6
	4—V	0	0	0
	4—H	0	0	0
Live Stimulus G_3	7—V	0	4	0
	7—H	0	4	1
	8—V	0	1	0
	8—H	0	11	0
	9—V	0	7	0
	9—H	0	5	12
	10—V	0	6	4
	10—H	0	7	0

*S's with the same numerals were raised in pairs; V, vertical stripe positive; H, horizontal stripe positive.
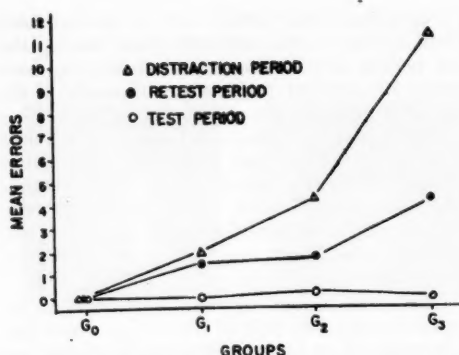
Fig. 4. Error scores during the experimental periods.

ordered alternatives (1954) was applied to the data. This test evaluates the null hypothesis that the samples are drawn from the same population. If the null hypothesis is rejected, this test gives support to the alternative in question, in this case that the rank order of group error means is $G_1 < G_2 < G_3$.

Although a monotonically increasing function appeared in both the distraction and the retest periods, the null hypothesis could only be rejected in the former case ($p = \cdot023$). Thus, as the distracting stimulus approached the social level, it became more effective. Since the gradient of distraction could not be statistically demonstrated during the retest period, the distracting effects can be considered transient.

Qualitative Observations. The quantitative data alone does not adequately describe the behaviour exhibited during the distraction period. The modal behaviour pattern in each group will be summarized.

G_0. All S's ran the maze without hesitation. The behaviour was not distinguishable from that exhibited during the test period.

G_1. All S's stopped and sniffed at the cardboard "rat" during the first few trials. The one rat (1—V, Table I) which produced all of the errors for this group showed no evidence of attending to the distracting stimulus on the trials in which it made an error. It passed the cardboard rat without hesitation and went through the incorrect stimulus door.

G_2. All S's showed signs of responding to the toy "rat" during the first few trials. They hesitated and followed the distracting stimulus to the incorrect door but did not go through. The sudden noise from the tin wheels as the distracting stimulus was pulled through the doorway produced retreating behaviour in a few cases. S's 3—V and 3—H made all of the errors. In practically all of the trials in which these rats did not follow the distracting stimulus, they completely ignored it.

G_3. Both observers agreed that the behaviour exhibited by this group was much more complex than that observed in any of the others. Plate II shows a sequence of pictures taken during a pilot study under social distraction conditions. The pictures were taken in the last section of the unmodified maze used in the training period (see Fig. 1). Although this section did not contain the glass wall and glass partition used in the experimental periods, the behaviour shown was typical of trials in the distraction period on which following occurred. Both rats came out of their starting boxes and nuzzled each other before the leader dashed through the maze with the follower immediately behind. This hesitation dropped out during the later trials, the leader running through immediately.

Two more interesting forms of adaptive behaviour were noted on some of the errorless trials. On 18 trials, both rats started in the wrong direction but reversed before they made an error. On 11 of these trials, they hesitated and sparred for position in much the same way that humans do when approaching another on the sidewalk. On the seven remaining trials they met in one of the narrow alleys; when this occurred, the rat heading toward the stimulus door invariably jumped over the other one and continued on its way.

Discussion

Stimulus Factors

Our results suggest a positively accelerated gradient, but three data points are not enough to consider the continuum of stimulus complexity established. All that may be said at the time is that there appears to be some monotonically increasing function between simple distraction and the complexity of the distracting stimulus.

We have used the term complexity to describe the change along a gradient of social stimulation. Further consideration of the distracting stimuli used in this experiment suggests a more analytic approach to the definition of complexity.

First, our limiting case, the live rat. A rat in a maze may be described as a moving white object, offering visual, auditory, olfactory, and occasional tactile cues to another rat. The toy dog may see similarly described and the assumption

seems warranted that, whatever its odour and sound, these would be more constant than those from the live rat and certainly not very rat-like. Finally, the cardboard rat may be considered a staionary white object, giving only visual and possible tactile cues.

These considerations suggest that the gradient of complexity is a multi-dimensional affair, including at least movement and the number of activated sense modalities.

Some interesting ideas in Krech & Crutchfield's text (1948) suggest the identification of some other dimensions. They point out that, ". . . there is only a continuum from simple (non-social) to complex (social) fields" (1948, p. 8). In elaborating on this, they point out that, " . . . person objects differ from other objects in an individual's field, and are especially important in determining behaviour, because they have, among other characteristics, the properties of mobility, capriciousness, unpredictability . . . " (1948, p. 9).

These three properties seem useful as descriptive terms to help identify another way in which the intermediate stimuli used in this experiment differed from the live rat and from each other. The white cardboard was in the same place on every trial and served as a relatively constant cue. The toy dog was less constant in regard to its starting position, the speed with which it was pulled across the apparatus, and the noise made by its wheels. The live rat was still more inconstant in the cues it presented. The mobility, capriciousness, and unpredictability were especially apparent in our observations of the interaction between the subject rats and distracting stimulus rats in G_3.

Further work is needed to define the dimensions suggested by the above analysis. The method of distraction used in this experiment seems to offer a sensitive and reliable dependent variable for work along this line. The major problem will be the further analytical and physical analysis of the stimulus situation. Although we have used conditioned rather than innate behaviour as our response unit, the logic of using subjectively selected mechanical models as distracting stimuli is somewhat similar to that used by Tinbergen (1951). We would suggest that in the long run a more fruitful approach to a quantitative definition of the stimulus in this work will be the type of analysis and methods of stimulus control developed by Johanssen (1950) and Michotte (1954) in their work on event perception.

Motivational Factors

Most of the work on social behaviour in rats has indicated that social modification of behaviour is most easily shown when the social stimulus has been systematically associated with some form of reinforcement (Church, 1957a; 1957b; Miller & Dollard, 1951; Holder, 1958). In our work the effects of competition and aggression were eliminated and primary reinforcement was held constant. Thus, it is unlikely that these factors were effective. The fact that our S's were paired in the home cages, however, suggests that the gradient of behaviour change could be interpreted in terms of the generalisation of secondary reinforcement effects originally built up in the home cages. Further work in which previous social experience is varied can evaluate this hypothesis.

Summary

Rats were trained on a vertical vs. horizontal stripe discrimination with hunger drive and food reward. After a very stable level of performance was reached, both stimulus doors were unlocked and various distracting stimuli were introduced in the vicinity of the previously incorrect doorway. Three distracting stimuli were used: (a) a stationery white cardboard rat, (b) a small toy dog moving through the incorrect doorway, and (c) a live rat moving through the incorrect doorway.

All three distracting stimuli increased errors. The distracting effects were monotonically related to the complexity of the distracting stimuli, increasing in effectiveness from the cardboard rat, to the toy dog, to the live rat.

The results are interpreted in terms of the increasing complexity of the distracting stimuli. Two dimensions making up this gradient of complexity are suggested; the amount of stimulus movement and the number of activated sense modalities. Krech & Crutchfield's suggestions that a continuum of social stimulation should vary in mobility, capriciousness, and unpredictability are also applied in discussing the results.

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THE ECHOLOCATION OF FLYING INSECTS BY BATS

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When bats are hunting insects they adjust the pattern and tempo of their high frequency orientation sounds in a way that seems quite appropriate for active echolocation of small moving targets but distinctly unsuited for the passive detection of insects by listening for their flight sounds. (Griffin, 1953; 1958). The most obvious change is a marked rise in the pulse repetition rate just as the bat closes in on its prey. For example, *Eptesicus fuscus* often emits only four or five pulses per second in straight cruising flight, with silent intervals of 200 milliseconds between them; but during insect pursuit the same bat may shift to a "buzz" in which the pulses are separated by only five milliseconds. Similar flight patterns and vocal responses can be elicited by tossing into the vicinity of a hunting bat imitation insects such as pebbles or plagets of wet absorbent cotton. What little sound these make can scarcely resemble that of a real insect.

Yet this type of evidence is indirect and not wholly satisfactory, since some bats certainly do respond to the sounds made by buzzing insects (Moehres, 1950; Kolb, 1959). Studies of insect pursuit behaviour in the laboratory have recently enabled us to make four significant extensions of earlier studies under natural conditions: (1) a more detailed description of the bats' hunting tactics, (2) measurements of the high rates at which small insects are captured, (3) estimates of the minimum distances at which they are detected, and (4) selective masking experiments with low and high frequency noise which demonstrate conclusively that at least under some circumstances insects such as *Drosophila* are intercepted by echolocation.

It is convenient to distinguish three phases of insect hunting behaviour which appear always to be present with varying degrees of distinctness in bats of the family Vespertilionidae. The first or *search phase* is fairly straight flight in which the pulse repetition rate is relatively low, although the bat is evidently ready to attack flying insects. The second or *approach phase* begins when the bat first reacts to an insect, either by turning towards it, by increasing the pulse repetition rate, or both. Here the interval between pulses shortens progressively though often irregularly, as the bat apparently locates its prey more accurately and flies toward it. A *terminal phase* ensues when the bat is fairly close to the insect and emits a burst of pulses at a very high rate, the buzz. A similar buzz is emitted on dodging small wires or just before landing, but the buzz of an insect catch seems typically to last longer and to include more closely spaced pulses. In *Myotis lucifugus*, the species we have studied most thoroughly, the search phase involves pulse-to-pulse intervals varying from about 50 to 100 milliseconds or more; in general the larger the room the longer the intervals in this phase. The approach phase has intervals between about 50 and 10 milliseconds, and in the buzz the pulses are separated by only 4 to 7 milliseconds. Intervals of more than 50 milliseconds are thus characteristic of cruising or search, 50 to 10 milliseconds approach, and less than 10 milliseconds the terminal phase of very close pursuit or actual capture of the insect.

Methods

Although small insects are apparently detected and pursued one at a time, the bats we have studied were much more likely to begin active hunting when large numbers of insects were on the wing. Even at best we have found that only a small fraction of the *Myotis lucifugus* released in rooms filled with flying insects undertake serious hunting; many will slowly starve and would doubtless die eventually in a room where other individual bats catch hundreds of insects every day. Two or three sessions of a half-hour or less on different days are usually sufficient to find the good catchers, provided that the bats are wide awake, warm, and active when tested. The 90 per cent. or more that did not show signs of hunting after several such sessions were released.

A good catcher identifies itself by making obvious interception manoeuvres, turning sharply, suddenly climbing or diving for a few inches, or merely by pulling up abruptly in the midst of fairly straight flight. Often the interfemoral membrane can be seen to turn upwards and forward, and the head may be thrust down into it very rapidly and out again. If the room is quiet, one can hear with the unaided ear the

faint audible clicks which accompany each pulse of high frequency sound during the search or approach phases. The buzz accompanying the actual catching manoeuvre is usually more clearly audible. With adequate lighting the actual capture of insects may be seen, but this has been much less obvious than the flight manoeuvres or the faint audible buzzes.

We have studied insect hunting in three different rooms, all about 8 feet high. The first, at the Vero Beach Laboratory of the Florida State Board of Health, was 8 ft. × 16 ft., the two used later in Cambridge, Massachusetts, were 12 ft. × 32 ft. and 11 ft. × 16 ft. All of these rooms had walls which reflected high frequency sound quite well, and in all cases the bats must have received strong echoes from walls, ceiling, and floor along with the much fainter echoes from their insect prey. This "clutter" may well have been what discouraged most bats from hunting indoors.

Sufficient numbers of insects might be obtained by light traps or other means of attracting wild insects, but we have reared mosquitoes and fruit flies by standard methods (Galtsoff, *et al.*, 1959). It was necessary to stir the insects into flight every few minutes in order to elicit continuous hunting. The actual densities of flying insects varied widely, both in space and time; but the great majority of catches were probably made at densities of no more than three or four insects per cubic foot.

Since insect capture by bats is a rapid process, and since the small insect is often very difficult to see at all, we were anxious to record the hunting behaviour by single photographs and motion pictures. With single photographs, even when the insect as well as the bat is shown, it is difficult to be certain what part of a complex sequence of events is recorded. Multiple flash stroboscopic photographs have been very useful, and at a flashing rate of five to ten per second the successive images of a flying bat are sufficiently separated, except in turns or hovering manoeuvres. Insects can be photographed better with multiple than with single flashes, because a row of specks equal in number to the flashes is less likely to be confused with dust on the film. We have used both still and motion picture cameras in pairs, spaced 0·5 or 1 metre apart, in order to obtain more accurate information about the positions of bat and insect, by simultaneous cross bearings.

In many experiments a tape recording was made of the bat's orientation sounds with a plastic dielectric condenser microphone (Kuhl *et al.*, 1954), a suitable amplifier, band pass filter, and an Ampex tape recorder. Each flash of a strobe light can easily produce a recognizable electrical signal on the tape, so that when oscillograph pictures of the bat's sounds were later prepared from the tape recordings it was quite feasible to identify each flash and determine its time of occurrence relative to the recorded sounds from the bat.

Illumination and background surface are of great importance because bats are very dark and tend to blend into most backgrounds if photographed by reflected light. It is relatively easy to obtain silhouettes of flying bats against a white background, and if the background is marked off with a grid of contrasting lines, the bat's position can be determined with one camera by making parallax corrections (Grinnell & Griffin, 1958). If two lights are employed, the separation between the two shadows of a bat can be used to determine its distance from the wall. We were not able to use this method to photograph mosquitoes or *Drosophila*, however, and turned instead to a very dark background of black velvet with lateral illumination either by flood lamps or an electronic flash. By careful arrangement of the lamp or lamps it was possible to photograph both bats and individual fruit flies on 16 mm. film. The flies appeared only as specks, but in many cases a careful study of the film with a time motion study projector permitted unequivocal identification of the flies by their characteristic movement. Catches were recognized by the approach of a bat to a particular fly followed by the disappearance of the latter as the bat flew on. A dust speck could easily be mistaken for a single image of a fly, but dust varied randomly from frame to frame while flies remained in approximately the same place or moved progressively. Furthermore dust specks were not intercepted by the bats.

1. Hunting Tactics

Most of the pursuit and interception manoeuvres requires less than one second and are extremely difficult to observe directly. We therefore photographed the bats and insects and recorded simultaneously the pattern of the orientation sounds. Each of the catches for which full or fragmentary sound and position records were obtained was preceded by the customary search and approach phases defined above, although there were some catches or apparrent catches of mosquitos without a full-fledged buzz. In such

cases the pulse-to-pulse interval dropped from about 90 milliseconds in the search phase only to about 40 or 50 milliseconds. This shift from search to approach phases provides the best indication yet available of the distance by which a bat has already detected some small object, such as a wire or an insect. In two mosquito catches for which excellent records were obtained there was a clear drop in this interval at about 100 centimetres and at about 30 centimetres respectively from the probable position of the mosquito. That is, the interval showed a marked drop at these distances from the spot where, a fraction of a second later, the bat was to emit its sharp buzz. Mosquitos can therefore be detected at appreciable distances, but further discussion of this topic is best deferred to Section 3.

The manner in which the frequencies within each pulse varied during these catching manoeuvres is also of interest. In the search phase, before any apparent reaction to the insects, the pulses were about two milliseconds in duration, the intervals between pulses were roughly 80-100 milliseconds, and the frequency dropped in each pulse from about 75-85 kilocycles at the start to 37-45 kilocycles at the end. This is the typical octave of frequency sweep in a *Myotis* pulse, although the actual frequencies were slightly lower than on some other occasions. During the two pronounced buzzes mentioned above the pulse durations fell to 0·5 milliseconds, or sometimes a little less. The estimated frequencies within these and other very short pulses ranged from about 25 to 30 kilocycles. Our recent and technically much improved records thus require an important modification of the first description of frequency patterns during insect catching (Griffin, 1953). It is now clear both for *Myotis* and *Eptesicus* during the pursuit of real and imitation insects that in the terminal buzz the frequencies are distinctly *lower* than in the search phase (previously referred to as "cruising", an appropriate term for *Eptesicus* under natural conditions but much less apt for *Myotis* catching insects at high rates in a small space).

During the pursuit of an insect the actual lengths of the pulses of sound in air drop, in proportion to the pulse duration, from about 70 cm. to 15 cm. The distance travelled by the bat between pulses varies with its flying speed, but before and just after detection (indicated by the drop in pulse-to-pulse interval) the bats were flying at 2 to 3 metres per second, or 2 to 3 millimetres per millisecond. One of the longer intervals thus represents 200-300 mm. of travel, while during the two clearest buzzes the bat's speed was about 70 cm./second or 0·7 mm./millisecond. Since one pulse was emitted every 5 to 7 milliseconds, the bat was now travelling only about 3·5 to 5 mm. between pulses. The velocity of sound in air is about 340 mm./millisecond, so that the wave lengths of the orientation sounds were about 4 to 8 mm. in the search phase, but increased during the buzz. The catching manoeuvres and their relationship to the pattern of the buzz will be described in more detail in a later paper.

2. Rates of Insect Capture

a. Mosquitos

In April, 1958, at the invitation of E. T. Nielsen several bats were brought to his 8 ft. × 16 ft. flight chamber at Vero Beach, Florida, which contained initially about 2,000 mosquitos (*Culex quinquefaciatus*). Several *Myotis lucifugus*, one *M. subulatus*, one *M. subulatus leibii*, two *Pipistrellus subflavus*, and two *Plecotus rafinesquii* were set free in this room for 10-15 minutes at a time, and certain of the *Myotis* hunted actively. These were left in the chamber overnight, and in the morning the mosquito population had fallen to roughly 200-300. Although none of the *Pipistrellus* or *Plecotus* was ever observed to hunt actively, a few of the *Myotis* were so successful that we could estimate the rate of insect capture not only by counting audible buzzes, but also by measuring the rate at which the bats gained weight (See Table I). Many factors might reduce the weight gain—evaporation of water from skin or lungs, urination or defaecation—but since the bats were closely watched we could be sure that they did not eat or drink anything but insects caught on the wing. Their gain in weight thus represents a conservative minimum estimate of the weight of insects captured.

It was not practicable to restore the population of mosquitos to its original level during these experiments, and on some of the subsequent days only a few hundred were present in the flight chamber. Yet in eight cases listed in Table I accurate weighing demonstrated high rates of mosquito catching. On other occasions the same bats gained very little, or even lost weight while flying in the same chamber. In these cases observation and listening for the faint audible components of the buzzes confirmed that they were hunting much less actively. The mosquito population was so low on 5th April

Table I. Rates of Mosquito Catching by Bats at the Vero Beach Laboratory, April 1958. The average weight of six of the mosquitoes used (*Culex quinquefaciatus*) was 2·2 milligrams.

Bat	Date	Duration of flight (minutes)	Weight gain (mg.)	Mosquitoes caught per minute
M. lucifugus Number 5 (Wt. 5·7 gr.)	April 1	30	350	5·3
	April 4	31	100	1·5
	April 4	9·5	73	3·5
	April 5	15	55	1·7
M. lucifugus Number 7 (Wt. 5·7 gr.)	April 2	10	125	5·7
M. subulatus leibii (Wt. 3·7 gr.)	April 2	21	77	1·7
	April 4	24	264	5
	April 5	15	312	9·5

that it was supplemented by releasing in the same room several dozen large crane flies (*Brachryprenna*). These weighed about 19 mg., but the bats usually dropped the legs and wings uneaten, and the bodies weighed only about 12 mg. During the 15 minutes when the *M. subulatus leibii* was observed to gain 372 mg. it was clearly observed to catch no more than 4 or 5 crane flies along with many mosquitos. It seems most likely that less than 60 mg. of its 372 mg. weight gain consisted of the crane flies, so that its rate of mosquito catching is listed in Table I as 312/15 or 21 mg. per minute. This bat was thus catching about ten mosquitos per minute or one every six seconds.

b. *Drosophila*

During the spring and summer of 1959 large numbers of *Drosophila* were reared in an 11 ft. × 16 ft. flight chamber and out of many *Myotis lucifugus* which were allowed to fly in this chamber, four, designated below as TR, S, B and TL, began active catching immediately when first allowed to fly in this room. The fruit flies were unfortunately not maintained in pure culture, but only two species were present, *Drosophila robusta* weighing 3 mg. on the average, and *D. melanogaster* with an average weight of 0·6 mg. On several occasions certain of these bats gained as much as 29 mg./minute while catching only fruit flies in this room. Sustained catching rates of 20 fruit flies per minute were observed, and two flies were often taken within one second (For details see below,

especially Table III). These rates of weight gain were in satisfactory agreement with the number of visible pursuit manoeuvres and audible buzzes which were often noticeable every few seconds, and are also quite similar to the estimates reported by Gould (1955; 1959) for the same species hunting under natural conditions.

3. Distances at which Insects are Detected

a. Procedures

This subject was studied by a minor modification of the photographic method used to measure the distance at which small wires are detected (Grinnell & Griffin, 1958). Bats and fruit flies were photographed in an 11 ft. × 16 ft. flight room with bright lateral illumination against a dark velvet background with a 16 mm. Auricon sound-on-film camera. The bat's orientation sounds picked up by the microphone were rectified into low frequency clicks by a detector circuit, and supplied to the input of the sound camera. Each bat pulse was registered, on the same piece of film as the pictures, as a click which contained no information about the original frequency, only rough information about pulse duration and intensity, but accurate information about pulse repetition rate or interval between pulses. This camera operates at 24 frames per second, with its shutter open about 1/50th second. While the images were small and slightly blurred by the bat's motion, they sufficed to show its position within one or two inches every 24th of a second. Actually the

position shown was the projection of the bat or fly against the opposite wall, and estimates of the distance between the two are subject to parallax errors in either direction. The apparent distance was greater than the actual distance when bat and fly were equally far from the camera, but less when one was behind the other. We could make only rough estimates of the bat's distance from the camera or the opposite wall, and hence we did not attempt to apply parallax corrections. But a long series of such single camera pictures allowed approximate estimates of the distance of detection and provided a most helpful background for more accurate determinations of bat and insect positions with paired cameras.

Detailed analyses were made of 34 catches or attempted catches by one *M. lucifugus*, S, 26 by another of the same species, B, and six by a *M. keenii septentrionalis*. In each of these 66 cases graphs were prepared showing, on a common time base, the pulse-to-pulse intervals, the vertical and horizontal positions of the bat and fly, and the apparent distance between them, that is, the distance between their images on the wall opposite the camera.

b. Special Aspects of Insect Pursuit Behaviour Relevant to Measurements of the Distance of Detection

Before proceeding to analyse these data on distance of insect detection it is important to review certain aspects of the bat's behaviour during these insect catching manoeuvres. In several cases the bat began its pursuit by taking off from the wall where it had previously been resting, and flew out to catch a fly which may well have been detected before take-off. In many other instances, however, the detection clearly occurred while the bat was flying; often many flies were caught during a continuous flight; and sometimes the bat did not land until hundreds of catches had been made. When starts from the wall were eliminated along with cases where the bat was turning away from the wall, catching another insect, or executing some other special manoeuvre at the start of the search phase, the pulse-to-pulse interval during search varied from 48 to 85 milliseconds, with the range from 50 to 60 milliseconds predominating. On the average the first clear drop below 50 milliseconds occurred about 0·5 second before the catch; and after that time almost every approach phase included a steady drop in interval. In all cases the actual detection must have taken place some-

what earlier than the vocal reaction of shortening the pulse-to-pulse interval. But since we have no way of estimating the bat's reaction time we will conservatively consider that detection occurred at the moment when the first pulse was emitted after an interval significantly shorter than that characteristic of the search phase.

The actual number of pulses emitted during the approach phase varied between 4 and 22; the average for the two *M. lucifugus* was 8·1 and for the *M. keenii* 10·6. These pulses are presumably used to track the moving insect. The terminal buzz contained anywhere from 3 or 4 to 15 or 20 pulses separated by 5 to 10 milliseconds. We have no way of determining whether the last pulse of the search phase served for detection, or whether several searching pulses at roughly 50-millisecond intervals were required. The number cannot be very large, however, because of the numerous cases described in the next section in which one catch was completed and a second detection and interception begun within a remarkably short period of time.

c. Double Catches

In six of the 66 cases selected for careful study there were two catches or attempted catches within less than one second. The actual period between catches was ¾ second for the *M. keenii*, ½ second for *M. lucifugus*, and three cases of about ½ second plus one of ¾ second for *M. lucifugus* B. In all cases the two flies were intercepted by distinct manoeuvres, and it seems probable that the second insect was located only after the first had been seized. (In one of these six cases the first fly was attacked but missed). Hence only a short interval of time and a few pulses are necessary for a successful search phase and detection. One of the clearest examples of double catches within one second is illustrated in Fig. 1. The positions of bat and fly were not clear until after the moment when the sound track first became adequate to show the tempo of the orientation sounds, but the latter part of the first approach phase was clearly photographed. The most significant aspect of this sequence is the time between the two catches. The next pulse to follow the first buzz came at a long interval (118 milliseconds) after the last pulse in the buzz. This is a common feature of these records, and it may well represent the time needed to remove the insect from interfemoral membrane where it is often "pouched" immediately upon capture or which it may strike before being seized in the teeth.
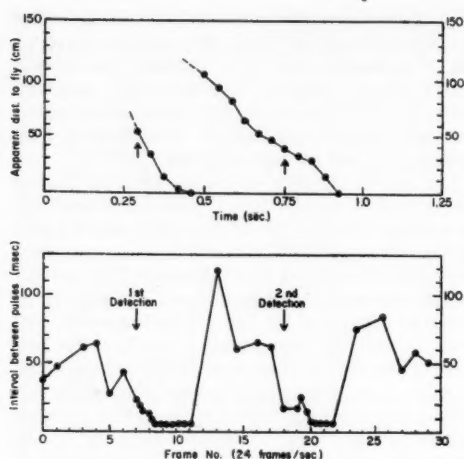
Fig. 1. A typical double catch within half a second. The upper graph shows as a function of time the apparent distance between the bat and the two *Drosophila*, that is, the separation between their images against the wall opposite the camera. Parallax errors may render these distances too high by 25 per cent. Each point on the lower graph shows on the same time base the interval separating one pulse of sound emitted by the bat from the previous pulse. Arrows mark the first vocal reaction to each fly, conservatively designed as the point of detection. During the two buzzes there were more pulses than could be plotted as separate points. Note that only four pulses were emitted in the search phase for the second catch (between 0.6 and 0.7 second).

Then three pulses were emitted at intervals of about 62 milliseconds, a value quite similar to the search phases just before and after the two catches. The next pulse came at an interval of only 17 milliseconds and clearly represented the beginning of the second approach phase. Only four pulses were emitted during the search phase between the two catches, hence the second fruit fly must have been detected by means of no more than four echoes. The reactions of these bats must thus be very rapid, and the information extracted from a very few pulses must suffice to locate the small target presented by a *Drosophila*.

d. Results Obtained with One Camera

Almost every one of the 66 cases of fruit fly catches analysed graphically provided a clear indication of the point at which the search phase gave way to the approach phase, thus demonstrating that the fly had already been detected. When the apparent distances of detection were averaged without parallax correction, the values were 73 cm. for the *Myotis keenii*, 55 cm. for *M. lucifugus* S, and 73 cm. for *M. lucifugus* B. The extreme values of these estimated distances of detection were 21 and 135 cm. Our next step was to average the individual values of pulse-to-pulse interval as a function of apparent distance from the fly. The mean value of the interval thus obtained showed an increase out to a distance of about one metre. This might be interpreted as evidence that detection occurs at this distance, but the flight patterns and other circumstances such as proximity to the walls differed so much in these several cases that average curves are of somewhat doubtful significance. We therefore selected 16 cases where parallax errors seemed minimal, when the bat did not start from the wall, and when the search phase was relatively uncomplicated by immediately previous manoeuvres. On averaging these for each bat, the mean pulse-to-pulse interval was found to increase rather smoothly to reach a plateau value at 70 cm. for *M. lucifugus* S, 55 cm. for B, and 90 cm. for the *M. keenii*. Parallax errors render these values too high by perhaps 20 or 25 per cent. but detection at half a metre would appear to have been common, and an occasional detection may well have occurred at one metre.

e. Measurements with Two Cameras

After these records had been analysed we made a further effort to determine the distance of detection more accurately by using two 16 mm. motion picture cameras to photograph many *Drosophila* catches by two *M. lucifugus*, TL and TR, which were two of the better catchers under our experimental conditions. Both were Auricon sound-on-film cameras operated by synchronous motors at 24 frames per second. Each received the same input of rectified pulses from the microphone, and each photographed a clock with minute and second hands. To aid in correlating the two films, the field of each camera also included a clock hand that revolved once per second and an oscilloscope with the horizontal sweep set at 20 per second. Careful study of several hundred feet of paired films permitted the selection of 11 clear cases in which a *Drosophila* catch could be unequivocally located on both films, i.e. both bat and fly were depicted throughout search, approach, and terminal phases, and there was no doubt concerning the matching of individual frames of the two films. In each pair of pictures the bat or fly appeared against the different spots on the grid on the opposite wall, and lines could be drawn on a

floor plan of the room from the positions of the camera to these projections on the wall. The intersections of these lines located bat or fly within one or two inches. The same accuracy could equally well have been achieved in the vertical position, but in all eleven cases the bat and fly were either flying almost horizontally, or were diving or climbing too slowly to introduce appreciable errors when vertical movements were ignored.

For these eleven cases we thus obtained fairly precise plots of the positions of bat and fly every 24th of a second, and from these we could measure the distance from bat to fly. We could then plot the pulse-to-pulse interval on the same time base as the distance separating the bat from
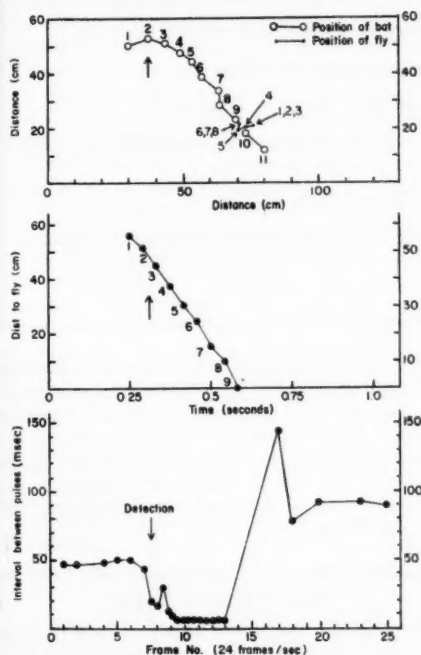

Fig. 2. Positions of bat and fruit fly and the pattern of orientation sounds during the catch. The upper graph shows a floor plan of part of the flight room some distance from the walls in which the zero point is arbitrarily selected to show the bat's position, and that of the fly, as determined by cross bearings from two motion picture cameras. The middle graph shows the distance separating bat and fly, and the numbers on the upper graph and middle graphs refer to frames of the film. The lower graph shows, on the same time base as the middle graph, the intervals between pulses. The arrow indicates the assumed point of detection. As in Fig. 1 the buzz contained more pulses than can be depicted individually.

its target. Three samples of the resulting combined graphs are shown in Figs. 2, 3, and 4; these are representative, although they include more clear-cut shifts from search to approach phase than some of the other cases. All eleven cases permit fairly accurate estimates of the distance of detection, however, and these distances are listed in Table II, along with the angular position of the fly relative to the bat's flight
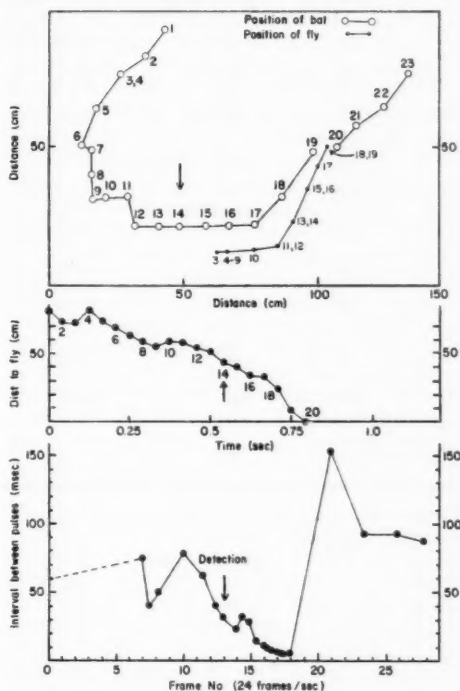

Fig. 3. Positions of bat and fly, together with the pattern of sound emission in another catch manoeuvre. For details see legend for Fig. 2.

path at the time when the search phase ended and the approach phase began. It is quite evident that these more accurate measurements confirm the tentative conclusion reached above that detection commonly occurred at about half a metre. The shortest measured distance of detection was 23 and the longest 83 cm.

The motion pictures suggest that the bat turns towards its prey within a small fraction of a second after detection. It is also of considerable interest to note that many detections seem to have occurred at considerable distances even though the fly was not directly in front of the bat.

Table II. Distances and Directions at which *Drosophila* were Detected by Two *Myotis lucifugus*. Detection is assumed to occur when the pulse-to-pulse interval first drops below the level characteristic of the search phase. Direction is given relative to the bat's flight direction at the time of detection.

| Catch no.	Bat	Dist. of detection (cm.)	Direction of fly at time of detection (degrees)	Remarks
1	TR	62	15°L.	Bat had just turned away from wall, fly moving toward bat, detection may have occurred earlier, perhaps at 80 c.m.
2	TL	52	5°R.	Bat approaching at 90°
3	TL	68	60°R.	Approach from behind fly
4	TR	23	50°L.	Oblique approach from behind fly
5	TR	34	15°L.	Fly almost stationary, straight approach
6	TR	45	45°R.	Fly stationary, straight approach (Fig. 2)
7	TR	27	40°R.	Oblique approach from behind fly
8	TL	42	15°L.	Approach from behind fly (Fig. 3)
9	TL	83	35°R.	Fly approaching bat, detection may have been at about 90 cm.
10	TL	56	35°R.	Approaching at about 90° (Fig. 4)
11	TR	40	70°R.	Bat out of field of one camera during part of approach phase
Average distance of detection		48		
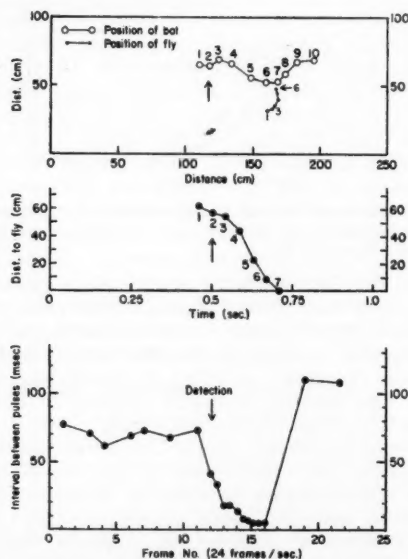
Fig. 4. Positions of bat and fly together with the pattern of sound emission in a third catch manoeuvre. For details see legend for Fig. 2.

Indeed there is little if any correlation between the angular position of the fly at the time of detection and the distance at which detection occurred. Since these bats usually seem to face in approximately the direction they are flying, at least in the search phase, this indicates that *Drosophila* can be detected from anywhere within a cone of perhaps 120°.

4. Selective Jamming Experiments with Low and High Frequency Noise

The evidence presented above suffices to show that small insects such as mosquitos and fruit flies are detected rapidly and at distances of about 50 cm. But even when taken together with the marked adaptation of the tempo of orientation sounds, this evidence does not prove conclusively that the insects are detected by means of echoes. It would still be logically possible to postulate that detection was achieved by hearing sounds of the insects' wingbeats. *Drosophila* were selected for the above experiments partly because their flight is very quiet, so that detection by passive listening would be more difficult than in the case of mosquitos or many of the larger flies and beetles

Furthermore, Williams & Galambos (1950) have measured the wave form of the flight sounds from a single *Drosophila funebris* beating its wings while attached to a small wire. The fundamental frequency under these conditions was about 180 cycles per second, and while the waves were complex enough to demonstrate the presence of strong harmonics, there do not appear to be any above the 10th at appreciable amplitude. The sound of a single *Drosophila* is very faint; Williams & Galambos used a 640AA condenser microphone at 1·5 cm. from the fly but did not estimate the absolute sound pressure level.

We have measured the sounds emitted by *Drosophila melanogaster* when 20-30 at a time were placed in a small glass bottle (about 50 millilitres) covered at the top by gauze. When the flies were strongly agitated and buzzing vigorously the r.m.s. sound pressure level was 25-30 decibels above 0·0002 dyne/cm.^{2} at 15 cm. from the gauze covered end of the bottle. This sound level, measured with a calibrated 640AA microphone, must have been considerably higher than that from a single fly in flight at 50 cm. from a hunting bat; probably the level reaching the bat's ears at this typical distance of detection would have been below 20 decibels. The observed frequency was between 500 and 800 cycles/second which is considerably higher than that measured by Williams & Galambos. It seems probable that resonance phenomena associated with the bottle may have accounted for this difference, but for present purposes the important point is that 20 or 30 flies, aided by the bottle's resonance, produced such a low sound pressure level.

If these bats detect fruit flies by hearing their flight sounds, it should be possible to mask these low frequency and low intensity sounds from the fly without employing a masking noise of high enough frequency to interfere with the bat's echolocation. Our best microphones when used together with an ultrasonic spectrum analyser could detect no ultrasonic components in the flight sounds of *Drosophila*, even though adequate to reveal sounds as faint as 25-30 decibels, above 0·0002 dyne/cm.^{2} We can thus exclude the posibility that *Drosophila* are detected by hearing ultrasonic components of their flight sounds, unless these components lie below this level of intensity. For selective masking experiments we therefore set up a simple apparatus to generate noise over the frequency band from

about 50-15,000 cycles/second. It consisted of a Grason-Stadler noise generator, a 20-watt high fidelity audio amplifier, and a combination of a standard 8-inch dynamic loudspeaker with an Electrovoice driver loudspeaker for the higher audible frequencies. This system filled the small flight room with a noise so loud that we had almost to shout when we wished to communicate. For comparison with this loud low frequency noise a rather weak thermal noise in the bat's frequency range was also used for certain experiments. It was generated by a General Radio type 1390A noise generator, a broad band amplifier which delivered into four Isophon electrostatic loudspeakers about 50 volts r.m.s. These loudspeakers gave a declining output above about 25 kilocycles/second, but still emit an appreciable signal to 55 kilocycles, and a weaker signal at least over much of the range from 55 to 100 kilocycles. For some experiments electronic filters were used to restrict the frequency band of the low or high frequency noise. The low frequency noise sounded roughly equal in intensity throughout the small flight room used for observations of *Drosophila* catching, but the intensity of the high frequency noise undoubtedly varied widely from point to point, and it was not measured. Measurements with a calibrated 640AA microphone placed the overall sound pressure level of the 100-5,000 cycles/second band of the low frequency noise at $+87$ decibels re 0·0002 dyne/cm.^{2} Its spectrum level did not vary greatly from 100-8,000 cycles/second as judged by ear and by measuring the level passed by a narrow band filter moved gradually across the frequency range from 100-10,000 cycles/second. The noise level was thus approximately 50 decibels per cycle bandwidth or 20-30 decibels more than the *overall* sound level of several *Drosophila* flying inside a small bottle.

When *Myotis lucifugus* which has previously been good fruit fly catchers were exposed to these two noises the results were consistent and unequivocal. The low frequency noise had no noticeable effect; the bats continued to catch flies as rapidly in this noise as in the quiet. The high frequency noise, however, produced a marked decrease in the number of pursuit manoeuvres, the number of buzzes, and in the rate at which the bats increased weight while hunting. They did not stop hunting altogether, but usually made many fewer attempts to catch fruit flies, and then landed. If the high frequency noise was switched off they would often resume

Table III. Rates at which *Myotis lucifugus* Captured *Drosophila* in Low and High Frequency Noise. Rates of capture are based on the measured rate of weight gain divided by an assumed average weight of 2 mg. for the fruit flies, and hence are minima. Bat TR was used July 2-27, 1959, and bat TL August 23-24.

Date and time	Conditions of noise and light		Interval be-tween weigh-ings (min.)	Rate of insect capture (flies per min.)	Remarks
July 2	0·05—5 kc.	dark	10	7·5	
July 18	0·05—5 kc.	light	10·5	11	
July 19	0·05—5 kc.	light	15	14·8	
July 20	0·05—5 kc.	light	12·8	4·7	
July 22 2:06	Quiet	light	12	6	
Jiuy 22 2:22	0·05—15 kc.	light	6·7	14·2	
July 27 1:40	0·1—15 kc.	light	3	13·5	
July 27 1:51	20—100 kc.	dark	3	—	Lost 1·7 mg./min.
July 27 2:00	Quiet	dark	3	11·7	
July 27 2:12	40—100 kc.	dark	3	1	
July 27 2:19	0·1—15 kc.	dark	3	3·8	Flies scarce
July 27 2:27	Quiet	dark	3	3·4	Flies scarce
Aug.23 2:32	0·1—15 kc.	light	6	13·7	
Aug.23 2:44	20—100 kc.	light	6	—	Lost 11 mg./min.
Aug.23 2:56	Quiet	light	6	9·3	
Aug.23 3:07	40—100 kc.	light	6	—	Lost 10 mg./min.
Aug.23 3:18	0·1—15 kc.	light	6	6·9	
Aug.23 3:31	Quiet	light	6	3·2	Bat tired
Aug.24 4:00	0·1—15 kc.	light	3	13·1	
Aug.24 4:13	20—100 kc.	light	4	—	Lost 29 mg./min.
Aug.24 4:24	Quiet	light	4	10·9	
Aug.24 4:32	40—100 kc.	light	4	—	Lost 7·5 mg./min.
Aug.24 4:41	0·1—15 kc.	light	4	10·1	
Aug.24 4:47	Quiet	light	4	7·8	Bat tired, flies scarce

hunting almost at once. Excluding measurements obtained when the bat appeared tired or when the flies were scarce, the average rate of *Drosophila* capture was 9·5 per minute in the quiet and 11 per minute in the low frequency noise. Nor was there any appreciable difference between experiments in the dark and in the light. The results of the several experiments summarized in Table III thus provide strong evidence against the possibliity that under these condit-ions fruit flies were intercepted by passive listening to their feeble flight sounds.

Discussion

When one watches a six-gramme bat chasing two-milligram fruit flies with dextrous, agile, and split-second manoeuvres, it is natural to wonder whether it obtains enough food from such tiny insects to equal metabolic energy required to catch them. Even the best catchers in our ex-

periments have required supplementary feeding of mealworms, but this was primarily because we could not keep enough fruit flies on the wing to furnish a normal night's catch. The metabolic rate of a flying bat has been estimated as roughly 100 calories per gramme per hour or 600 calories per hour for a six-gramme bat (Griffin, 1958). The caloric value of a *Drosophila* does not seem to have been measured, but a reasonable approximation is probably the value of 2241 calories per gramme listed by Trager (1953) for newly emerged adults of the fly *Ophyra cadaverina*. When our bats were catching ten fruit flies per minute (conservatively assumed to average 2 milligrams in weight) they were taking in food at a rate of roughly 2,700 calories per hour, provided the above assumptions are correct. Even though the supreme exertion of insect interception may require somewhat more than 600 calories per hour, the game does seem to be worth the candle.

Since *Myotis lucifugus* can detect echoes from *Drosophila* at 50 centimetres or more, it is appropriate to inquire what the intensity of such echoes would be. This will depend upon the sound level emitted by the bat, the frequency chosen for consideration, and the effective size of the fruit fly; but an approximate formula for the necessary computation is available (Griffin, 1958, p. 349). In the search phase the r.m.s. intensity of the outgoing pulse at 100 millimetres from the mouth of a *Myotis lucifugus* is about 100 decibels above the standard reference level of 0·0002 dyne/cm^2. This is the maximum value when the pulse is at its highest amplitude, and this peak amplitude occurs at roughly 60-70 kilocycles, so that the strongest component of the echo corresponds to a wave length of about 5 mm. For simplicity let us assume that the *Drosophila* returns an echo of 5 mm. sound waves equal to that from a rigid sphere of 2 mm. radius, a rough approximation but one that we cannot improve upon until actual target cross sections of insects for bat pulses are measured. Making the above assumptions one can compute that the echo from a *Drosophila* would have an r.m.s. value of about 30 decibels at a distance from bat to fly of 50 cm., approximately the distance by which our records show detection to have occurred. This calculation assumes that the inverse square law applies to both the emitted sound beyond 10 cm. from the bat's mouth, and to the echo from the fruit fly. It also takes into account the 2½ decibels of atmospheric attenuation at 68 kilocycles over a round trip distance

of 1 metre. If the fly were detected at one metre, rather than 50 cm. the echo would be reduced by sixteen fold, or by about 12 decibels. This, together with another 2½ decibels of atmospheric attentuation, would reduce the echo to 15 decibels, only slightly above the human auditory threshold at those frequencies where it is lowest.

Detection by bats of an echo having an intensity between 15 and 20 decibels appears plausible, except that the human auditory threshold of sound intensity rises sharply as the duration of a tone is decreased below about 200 milliseconds (Stevens, *et al.*, 1951, pp. 1,020-1022). These data suggest that a 1-millisecond pulse might have a threshold 100 times, or about 20 decibels higher than one lasting 200 milliseconds. Since 0 decibels is approximately the human threshold under favourable conditions, it does not strain credulity to postulate that a bat could detect one-millisecond pulses at 15 decibels especially since there is good reason to believe that the auditory brains of bats are highly specialised for the detection of short pulses of high frequency sound (Grinnell & Griffin, 1959).

Another important question concerns the choice of 68 kilocycles, or a wavelength of 5 mm. as the most easily detectible portion of the echo. Other factors being equal, higher frequencies or shoter wavelengths would return somewhat stronger echoes, but higher frequencies are not present at as high intensities in the emitted pulse, they suffer more atmospheric attenuation, and furthermore in the case of spheres with a 2 mm. radius the increase in echo is not very rapid between 68 and 120 kilocycles, the highest observed frequency in *Myotis* pulses. Lower frequencies are actually used during the interception phase, down to about 25 kilocycles in some cases. But of course the distance is then much shorter, and to a first approximation the echo intensity varies inversely as the fourth power of the distance. While the echo of a 25 kilocycles component would be weaker than that of 68 kilocycles by a factor of about six, the shorter distance would more than offset this.

These echoes which the bat seems to detect at a level of about 30 decibels may come from any direction within a cone of roughly 120°. Nor is mere detection enough for the hungry bat; it must localise the direction from which the echo is coming and execute an appropriate turn within a fraction of a second. The probing pulse of sound emitted by a *Myotis* has a rather broad angular spread, although the higher frequencies at the start of the pulse are more sharply con-.

centrated into the forward direction than the lower frequencies at the end. It is possible that in each of the cases shown in Table II where the angular position of the fruit fly was far removed from the flight path of the bat the animal happened to have its head turned in that particular direction at the moment of detection. But photographs of flying *Myotis* indicate that during the search phase the head is ordinarily pointed in the direction of flight, so that it seems more likely that echoes can be detected from anywhere within a fairly wide zone in front of the animal. The initial localization may not be precise, and the need for more accurate bearings may well explain the shortening of pulse-to-pulse interval during the approach phase. But an eventual understanding of the physiological mechanisms of echolocation must include not only the detection of insect echoes at a level of roughly 30 decibels, but their almost instantaneous localization as well.

Finally we should compare the difficulty of jamming the echolocation of small wires (Griffin & Grinnell, 1958) with the cessation of insect hunting in high frequency noise. It should be borne in mind that in the jamming experiments collisions with wires were presumably unpleasant enough to motivate their detection. But we have no way of ascertaining how strongly our bats were motivated to continue hunting when the noise was switched on. Insect hunting has been elicited only with difficulty, even in the quiet, and it is not surprising that high frequency noise would discourage it. In our jamming experiments with wires the bats preferred not to fly and they tried to avoid the difficult task of echolocating small wires in the noise. While we could make some of them do this by forcing them to fly in an array of wires, we have no comparable way of forcing bats to attempt insect catching in the noise. Furthermore, wires are larger in one dimension and are stationary, rather than moving targets, Bats often slow down on approaching wires, but insects are intercepted at flight speeds of at least one metre per second.

It is remarkable that our very loud low frequency noise did not reduce the rate of insect catching at all, and that the good catchers, TR and TL, caught approximately as many fruit flies in this noise as in the quiet. In human masking experiments low frequencies are found to mask higher tones more easily than the reverse, but the auditory system of a *Myotis* is clearly able to make sharp frequency discriminations (Grinnell & Griffin, 1959), and it must be able to

distinguish 30 decibel echoes at 60 or 70 kilocycles from a noise covering the band from 0·1 to 10 or 15 kilocycles at a level of about 50 decibels per cycle bandwidth.

Another question might be phrased as follows: since bats can apparently hear echoes from wires at a signal-to-noise ratio of −35 decibels (Griffin & Grinnell, 1958), why not assume that they can also hear *Drosophila* wing beats 30 decibels below a 50 decibel per cycle bandwidth noise at lower frequencies? This question leads directly into the problem of critical bands for the hearing of bats, a problem discussed elsewhere (Griffin, 1958, pp. 373-375). But regardless of the uncertainties surrounding this problem, it is difficult to believe that bat hearing or *Drosophila* flight sounds could have so narrow a bandwidth as one cycle per second. Hence the noise effectively competing with the flight sounds must be greater than 50 decibels. Even if we assume that the overall level of the *Drosophila* flight sounds (about 20 db. re 0·0002 dyne/cm^2) is spread over as narrow a bandwidth as 10 cycles/second, the competing noise would have a level of 60 decibels or 40 decibels above the sound level from the fly.

It should be reiterated that we are not suggesting that all detection of insect prey by all bats under all conditions is based upon echolocation. The observations of Moehres (1950), Kolb (1959) and others are sufficient to demonstrate passive detection of relatively noisy insects, and we have also observed bats suddenly respond to a buzzing blow fly and turn towards it from a distance of several feet. Quite clearly both active echolocation and passive detection are important in the feeding behaviour of bats under natural conditions. We have considered only the former, because of its intrinsic interest, and because its importance had not been fully recognized heretofore.

Further experiments now in progress seem likely to throw additional light on the intricate and rapid flight manoeuvres executed during insect pursuit and interception, the ways in which the various membranes are employed, and the accuracy of localization of the insects. These studies will be reported elsewhere, together with a consideration of the information processing involved in the tracking of small moving targets.

Summary

1. Bats of the genus *Myotis* (*M. lucifugus*, *M. subulatus leibii* and *M. keenii septentrionalis*) have been studied while pursuing and capturing

small insects under laboratory conditions. It is apparently important to provide fairly large numbers of such insects in order to elicit insect catching behaviour indoors.

2. Insect catches are individually directed pursuit manoeuvres; each insect is detected, located, and intercepted in flight within about half a second.

3. Certain individual bats caught mosquitos (*Culex quinquefaciatus*) and fruit flies (*Drosophila robusta* and *D. melanogaster*) at remarkably high rates which could be measured conservatively by the gain in weight of the bat. Sometimes a bat would average as many as 10 mosquitos or 14 fruit flies per minute during a period of several minutes. In four cases motion pictures showed two separate *Drosophila* catches within half a second.

4. The orientation sounds of the hunting bat are adjusted in a manner that seems appropriate for the echolocation of single insects one at a time. There is a *search phase* before the occurrence of any apparent reaction to the insect. In this phase the frequency drops from about 100 to 50 kilocycles during each pulse of sound, and the pulses are emitted by *M. lucifugus* at intervals of 50 to 100 milliseconds.

5. When an insect is detected the search phase gives way to an *approach phase* characterized by a progressive shortening of the pulse-to-pulse interval and, if necessary, a sharp turn towards the insect. In this phase the pulse duration may shorten somewhat, but the frequencies remain approximately the same as in the search phase or drop slightly.

6. When the bat is within a few centimetres of the insect there is a *terminal phase* in which the pulse duration and interval between pulses shorten to about 0·5 millisecond and 5 or 6 milliseconds respectively. Contrary to a conclusion reached earlier on the basis of much less adequate data (Griffin, 1953), the frequency *drops* in the terminal phase, sometimes to 25 or 30 kilocycles. This is the buzz, which also occurs in many cases when the bat is dodging wires or landing.

7. The distance from the insect at which detection occurs can be judged by the shift from search to approach patterns. This distance of detection is commonly about 50 cm. for *Drosophila*, and it occasionally may be as much as a metre with fruit flies or mosquitos.

8. Two *M. lucifugus* which had become adept at catching *Drosophila* in the laboratory were exposed to broad band thermal noise either at low frequencies (0·1-15 kilocycles) or high (20-100 kilocycles). The low frequency noise had an approximately uniform spectrum level of about 50 decibels per cycle band width (re 0·0002 dyne/cm^2) from 0·1 to 8 kilocycles. It was thus very loud compared to the flight sounds of *Drosophila* which have a fundamental frequency of a few hundred cycles/second and a maximum sound pressure level of 20-25 decibels at the distances of detection by these bats. The high frequency noise was of low and varying intensity, but it discouraged or prevented insect catching. The low frequency noise, on the other hand, had no effect on insect catching; the bats gained weight in this noise (and in the dark) just as rapidly as in the quiet. Although bats sometimes detect insect prey by passive listening to sounds emanating from the insects themselves, these experiments appear to us to establish conclusively that small and relatively silent insects are often detected by echolocation.

Acknowledgments

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THE RELATIONSHIP OF HORMONES TO THE REPRODUCTIVE BEHAVIOUR OF BIRDS, REFERRING ESPECIALLY TO PARENTAL BEHAVIOUR: A REVIEW

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Introduction

The following sources provide general reviews on endocrinology: Nalbandov (1953), Sturkie (1954), Breneman (1955), Marshall (1955), Turner (1955). Information which is generally accepted and for which I do not give detailed reference may be traced in these works.

The hormones mainly involved in reproductive behaviour are those of the anterior pituitary and the gonads. It seems evident in birds, particularly from observations on secondary sexual characters and rudimentary organs associated with the other sex, that the ovary secretes androgen as well as oestrogen, and that the testis also secretes both (see e.g. Domm, 1939; Noble & Wurm, 1940; Bullough, 1942). Progesterone has been found in the blood of both male and female birds, and there is evidence of its presence in the ovarian follicles of laying hens (Layne, Common, Maw & Fraps, 1957) and in the testes of male pigeons (Lofts & Marshall, 1959). Implants of bird pituitaries into other animals have shown the presence of both luteinizing hormone (LH) and follicle stimulating hormone (FSH). Prolactin is certainly secreted by the bird pituitary.

Since Riddle, Bates & Lahr (1935) found that prolactin influenced broody behaviour of hens, this hormone has been thought to control parental behaviour. One of the main contentions of this review is to suggest that prolactin is by no means as important to parental behaviour as has sometimes been supposed. Prolactin was first characterized by its effect of inducing milk production from the crop-sacs of pigeons and from mammalian mammary glands. Since then various additional physiological effects have been found. Prolactin was the first anterior pituitary hormone to be isolated in pure form, and its physical and chemical properties have been studied in detail, showing that it is a protein. General reviews on prolactin can be found in Pincus & Thimann, vols. 1 & 3 (1948 & 1955) and in White (1949). Various synonyms for prolactin are sometimes used, notably lactogenic hormone and luteotrophic hormone.

Another aim of this review is to indicate that not only do hormones influence behaviour but also that the behaviour may influence the pattern of hormone secretion. This point has recently been discussed by Lehrman (1959).

There are various problems which apply generally in the interpretation of endocrine experiments. A most important one is of the degree of directness by which any particular effect results from the treatment given; for instance, is a change in behaviour to be ascribed to a direct effect of the hormone upon central or peripheral nervous mechanisms, to its effect upon other specific organs including the sources of different hormones, or to general systemic effects. Such questions must be asked but are not easy to answer. One case of this problem which is very important to the present discussion is the relationship of progesterone to prolactin. Many effects which can be obtained by prolactin treatment can be obtained equally or better by progesterone treatment; in mammals it has been established that prolactin controls the secretion of progesterone, and therefore such a relationship probably also exists in birds and must be considered in the interpretation of experimental results. It is also necessary to relate the effects of experiments to the situation which occurs normally: it is clearly invalid to explain normal events on the basis of treatments which are inconsistent with the normal physiological state. Thus the question of dosage is of great importance, for many experiments have been made with super-physiological doses, and in some cases different doses of the same hormone have distinct effects. Synergism between different hormones appears increasingly to be important, and must also be borne in mind. It is therefore evident that even where there are clear experimental results, caution is necessary in their interpretation—and some of the available results on birds are not even clearly significant. Assays

of hormones present in animals are of obviously great importance, though even here caution is necessary as the quantity found depends not only on the rate of secretion but also on the rates of transfer to other organs and of breakdown.

A valuable adjunct or alternative to assay is cytological study of the endocrine organs. The cytology of the anterior pituitary is very complex and it is still not fully established how cytological changes correspond with changes in the pattern of secretion, and in particular which type of cell is the source of each of the hormones (see Martinet, 1952; Pearse, 1952). Wingstrand (1951) provides a review of the earlier work on birds, and there are more recent studies by Legait & Legait (1954) and Perek, Eckstein & Sobel (1957). Legait (1955) has also studied the cytology of the neurosecretory nuclei in the hypothalamus of the hen and its relationship to pituitary and reproductive activity. There is a rapidly increasing number of studies on the cytological changes in the gonads of birds, especially of males. Many of these cover only the gross seasonal changes of the beginning and end of the reproductive period, but a few attempt a more detailed correlation with behaviour (e.g. Petersen, 1955; Robinson, 1956; Marshall & Coombs, 1957; Davis, 1958).

In mammals, on which very much more endocrinological work has been done than on birds, marked interspecific variations have been found and generalizations may not be taken as universally valid. Undoubtedly there are also specific differences in the endocrinology of birds, a good example being provided by differences between the female turkey and the hen (van Tienhoven, 1958) which are comparatively closely related species. Since most work on birds has been done on the domestic fowl, whose reproductive behaviour is certainly abnormal, and on pigeons, which are exceptional in their possession of milk-producing crops, generalizations about the endocrinology of birds are especially dangerous. This review is therefore intended only as a framework upon which the design of future experiments may rest.

The Beginning of the Breeding Season and the Sexual Phase

In the late summer, when the breeding season is just over, the gonads of birds are regressed and the pituitary seems to be refractory to stimuli which cause response at other times (Marshall, 1955; Lofts & Marshall, 1958; Wolfson, 1959a). At this time the secretion of gonadotrophins, sex hormones and prolactin is probably minimal. Possibly autumn migration depends on such a state of quiescence (Bullough, 1945); however, the control of migration both in autumn and spring presents very complex problems which have been reviewed elsewhere (Farner, 1955; Wolfson, 1959b). In some resident birds the regression of the gonads may not be extreme (e.g. Williamson, 1956) and there is slight sexual activity during late autumn and early winter (Bullough, 1942; Morley, 1943; Marshall & Coombs, 1957) but a marked change still occurs in the spring. The spring time recrudescence of the gonads, upon which spring migration and the onset of full sexual behaviour depend, is the result of increased pituitary activity which appears to be largely a response to changing external conditions. There has been much study of the influence of photoperiod changes on gonad condition (see Burger, 1949; Marshall & Disney, 1956; Threadgold, 1958; Marshall & Serventy, 1958; Wolfson and Farner in Withrow, 1959), but although changes in daylength are certainly influential other factors are undoubtedly also of importance, especially in tropical birds which are normally subject to very little change in photoperiod (Marshall, 1951; Disney & Marshall, 1956; Marshall & Roberts, 1959; Marshall, 1959). Rainfall (e.g. Williamson, 1956), the availability of food and the presence of potential mates may all be important sources of influence. A very interesting set of experiments by Burger (1953) showed that testis growth in Starlings was much increased if the males were caged with females than if they were kept only with other males, but even so the testis size obtained was much less than that of normal wild birds. The effect of these factors upon the gonads is mediated by the anterior pituitary, there being ample evidence that pituitary activity is under neural control (Harris, 1955; Benoit & Assenmacher, 1955; Ganong, 1959).

The anterior pituitary secretes the gonadotrophins, FSH and LH. It is established that in birds, as in mammals, FSH stimulates testis tubule growth in the male and ovarian follicle growth in the female. In mammals FSH stimulates oestrogen secretion by the ovary, and this is probably also the case in birds although there is no good experimental evidence on this point. Bates, Lahr & Riddle (1935) found that administration of FSH led to growth of the hen oviduct, which is certainly influenced by oestrogen, although in conjunction with progesterone (see Lehrman & Brody, 1957). In hypophysectom-

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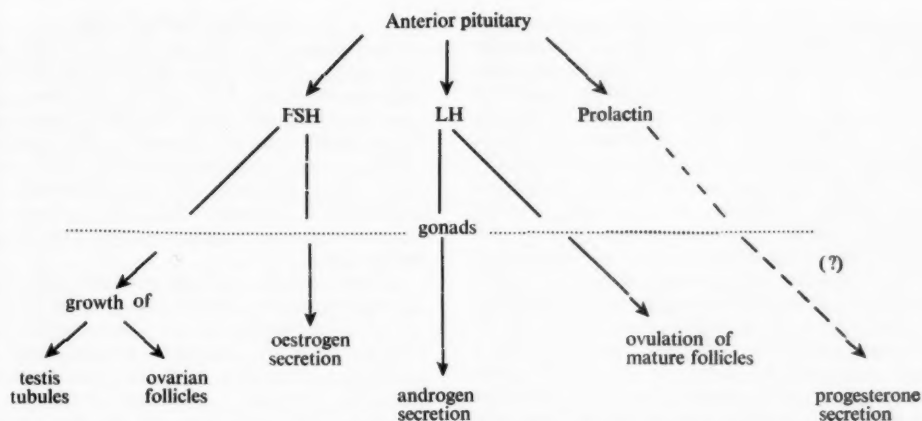d this
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Diagram to indicate the fundamental and well established relationships of the hormones to which reference is made. It is not intended as a complete representation and, in particular, does not show the reverse relationships between the gonadal and pituitary hormones which—though important—are not yet sufficiently well established in birds.

ized cockerels really pure FSH does not stimulate androgen secretion, as indicated by lack of comb growth; development and secretion of the testis interstitium is under the control of LH, and Riley & Fraps (1942a) and Fraps (1943) have shown that the pituitaries of male chickens have a high LH content. Androgen secretion is controlled by LH in mammals also.

Thus, under the influence of the gonadotrophins, the gonads recrudesce and the sexually quiescent phase comes to an end. Sexual behaviour is greatly influenced by androgen and oestrogen: this has been amply reviewed by Bullough (1945), Beach (1948) and Collias (1950). Seasonal changes in secondary sexual characters also often depend upon these same hormones (Domm, 1939): in *Pyromelana*, for instance, condition of the plumage always correlates with that of the gonads (Rollo & Domm, 1943). Aggressiveness and dominance in birds are clearly influenced by androgen but apparently not by oestrogen (Collias, 1950). Characteristic male vocalizations are also stimulated by androgen (Collias, 1950): Robinson (1956) found that the seasonal development of song patterns in the Australian Magpie could be correlated with the detailed changes in the state of the testis. Thus, the behaviour patterns characteristic of the early reproductive phase in the spring—that is song, fighting, the setting up of territory, and courtship—may be assumed to be controlled by the androgen and oestrogen released in increasing quantity by the recrudescent

gonads. These internal stimuli are of course operating in conjunction with stimuli from the environment, and also, as Lehrman (1959) discusses more fully, the behaviour itself probably results in an intensification of the hormone secretion.

It is often only after a distinct period of courtship that copulation first takes place, and this probably depends upon the attainment of full breeding condition. In the White-crowned Sparrow there is no perceptible interval between the attainment of breeding condition by the male and the first copulation (Blanchard, 1941), but in other species the male comes into condition before the female and copulation then depends upon her responsiveness (e.g. Tinbergen, 1939; Williams, 1952). It is a common observation that mating behaviour and nest-building are often very closely correlated in wild birds (Tinbergen, 1939; Emlen, 1941; Kuusisto, 1941; Nice, 1943; Williams, 1952), and such behaviour may coincide with the final and most rapid phase of ovular growth (Emlen, 1941; Petersen, 1955). On the basis of such observations of wild birds, combined with the studies of the gonad cycle (see p. 158), it seems reasonable to suppose that mating behaviour depends on comparatively high androgen and/or oestrogen levels, and that nest-building is also under the influence of these same internal factors. Until the elegant experiments of Lehrman which will be discussed below, there is little mention of nest-building in the literature of hormone experiments on birds.

Shoemaker (1939) treated female canaries with androgen and these birds then "frequently dangled a string or a piece of cotton before other females" but this is more probably courtship than strictly nest-building behaviour. Noble & Wurm (1940) found that androgen injections induced nest-building as well as characteristically male displays in Black-crowned Night Herons of both sexes, including even young chicks. Lehrman (1958a) found that if a nest bowl and nest material were given to a pair of Ring Doves which had been together in a cage for a week they would immediately engage in nest-building. If Ring Doves were paired after being kept in isolation and given oestrogen treatment for a week, they would similarly engage in nest-building (Lehrman, 1958b). Lehrman therefore suggests that participation in courtship stimulates oestrogen secretion, and that this in turn stimulates nest-building. There is no information available yet on the role of androgen in the nest-building of the Ring Dove.

At this time the incubation patches are developing (Nice, 1937; Bailey, 1952; Petersen, 1955; Johnston, 1956; Brackbill, 1958). The first change to occur is loss of feathers over the patches which, according to Bailey's experiments (1952), depends on the action first of oestrogen and then of prolactin. Unfortunately, Bailey did not test the effect of progesterone, which may well be important in this context.

The relationship in time between nest-building and the laying of the first egg is variable, not only between species but sometimes also within a species (Owen, 1959). However, at about the time of building the ovary and oviduct of the female increase greatly in size (Vaugien, 1955; Petersen, 1955). Hinde & Warren (1959) have shown in the Canary that laying is not related simply to the achievement of a nest, for birds permitted to build freely but not to achieve a nest laid at the same time as the controls, while laying was disturbed if the birds were prevented from building freely. The influence of nests and building upon later stages of the cycle is discussed more fully by Lehrman (1959). The exact time of laying of the first egg may be influenced by external conditions apart from those connected with the mate and the nest: Kluijver (1951) found in the Great Tit that four days after a rise in temperature in spring many clutches were started, but four days after a fall in temperature few were begun, and similar findings are reported by Myres (1955), Snow (1958) and Owen (1959).

The Egg-laying Period

Just before and during the egg-laying period the testes of male birds seem usually to be at a maximum of size and activity: in some species this stage of development may have been reached considerably earlier (Marshall, 1949; Johnston, 1956), in others the maximum size is not reached until about the time of laying (Petersen, 1955; Marshall & Coombs, 1957). There is no direct information on the hormonal state of the males at this time.

There is little enough relevant information on the hormone situation in female birds just prior to egg-laying. In the female mammal, the increasing FSH secretion induces increasing oestrogen secretion and the development of follicles. The rising oestrogen level then suppresses FSH secretion but induces increased secretion of LH until the time of ovulation. Prolactin secretion must also be induced by the rising oestrogen level (Meites & Turner, 1947) although concentrations of prolactin at this time would certainly not be comparable with those occurring during pregnancy and lactation. Prolactin secretion must also be postulated because prolactin controls the secretion of progesterone (see Folley & Malpress, 1948; Meites & Shelesnyak, 1957), and progesterone is secreted at this time and appears to be involved in the mechanism of ovulation (Sawyer, 1952, 1959; Forbes, 1953).

The situation in the female bird is probably similar, but there is much less information about it. Administration of FSH leads to growth of the oviduct, which is mediated by oestrogen and progesterone (see p. 156). Since the oviduct is maximally developed at the time of ovulation and laying (e.g. Vaugien, 1955; Petersen, 1955) the previous presence of these hormones must be presumed. Administration of oestrogen depresses FSH secretion in birds, as in mammals, so that the level of FSH has probably decreased by the time laying begins. In contrast to the evidence on mammals, oestrogen appears to depress LH secretion also in birds—at least in the cock, judging from effects on the testis and comb (Kumaran & Turner, 1949). That there may be such an effect normally in the hen is suggested by the findings of Fraps (1943) who assayed pituitaries by their power of inducing ovulation in the hen, which is much more effectively induced by LH than FSH (Fraps & Drury, 1943), and found that those of laying hens were slightly less potent than those of non-layers. Assays of pituitary tissues (Riley &

Fraps, 1942b) and blood (Bailey & Phillips, 1952) which do not differentiate between the two gonadotrophins show the least gonadotrophic potency in laying hens as compared with non-sexual birds; further Riley & Fraps (1942b) found an inverse correlation of gonadotrophic potency with oestrogen concentration as assayed from the droppings, those of laying birds being the more potent in oestrogen. Progesterone has been found in the blood and ovarian follicles of laying hens (Fraps, Hooker & Forbes, 1948; Layne et al., 1957; Lytle & Lorenz, 1958) but it is also present in the blood of non-laying hens (Fraps, Hooker & Forbes, 1949) and quantitative data are not yet available. There appears to be no relevant assay evidence on any bird other than the hen. Apart from the apparent difference in the influence of oestrogen on LH secretion, these data suggest a picture much like that of mammals.

There is corroborative evidence also from other sources. Lehrman's study of the Ring Dove (Lehrman & Brody, 1957; Lehrman, 1958 a & b) suggests the presence first of oestrogen and then of progesterone, while Bailey's observations and experiments (1952) on the brood patch of passerine birds suggest the presence of oestrogen and of prolactin. Whether there is a similar relationship between prolactin and progesterone in birds to that shown in mammals is not yet known, but again the concentrations of prolactin at this stage, just before laying begins, are certainly not as great as later in the reproductive cycle. This is indicated by Bailey's study (1952) of the brood patch, Lahr & Riddle (1938) on the crop-sac of pigeons, and studies of the hypothalamus and pituitary of the hen (Legait & Legait, 1954; Legait, 1955). At the time of laying in the female Sparrow, colour change of the beak indicates androgen release (Vaugien, 1955) which is also suggested at this time and during courtship in the female Night Heron (Noble & Wurm, 1940). This summarizes the available information on the hormonal state of the female bird just before laying begins.

The mechanism of ovulation in mammals has recently aroused considerable interest since the realization that the nervous system is implicated even in the so-called 'spontaneously ovulating' species (see Everett, 1959; Sawyer, 1959). Although ovulation, egg-formation and oviposition have been intensively studied in the hen, the problems of general biological interest involved have not been sufficiently emphasised. In particular, the serial ovulation shown by

birds presents a most interesting physiological problem which has now been discussed in general terms for the first time by Nalbandov (1959).

There is considerable evidence that ovulation in the hen depends upon a cyclic release of LH, occurring 6-8 hours beforehand according to Sturkie (1954) and 8-14 hours before according to van Tienhoven, Nalbandov & Norton (1954). It is established that ovulation can be induced by the administration of small quantities of progesterone in intact hens, but not in hypophysectomized hens, suggesting that the progesterone stimulates LH release. In addition, a drug which blocks LH release in mammals also blocked progesterone-induced ovulation in hens (van Tienhoven, Nalbandov & Norton, 1954). In mammals progesterone is released on the rupture of the follicle, and this may occur in birds also but as yet there is no evidence on this point. Rothchild & Fraps (1944) found that excision of the ruptured follicle produced failure of oviposition of the egg derived from that follicle, so that it is probable that the ruptured follicle regulates laying, but whether it has any influence upon the next ovulation in the series is not known. This next ovulation occurs shortly after (15-75 minutes in the hen) the laying of the previous egg, but it appears that the latter event does not directly control the former —which is, indeed, determined several hours earlier by the ovulatory release of LH. Such evidence as there is suggests, on the contrary, that oviposition may be in part determined by factors causing the subsequent ovulation (Bastian & Zarrow, 1955). However, though oviposition may have no influence upon the immediately following ovulation, the presence of the egg in the oviduct appears to be influential. It is known that ovulation does not occur while there is an egg in the oviduct, and this prompted Huston & Nalbandov (1953) to investigate the effect of an obstruction placed in it: they found that ovulation was inhibited for up to 30 days, while the condition of the gonads and comb showed that the basal secretion of FSH and LH must have been unaffected. Ovulation could be induced in these birds by injection of LH or small quantities of progesterone, and it was suggested that the normal ovulatory release of LH was neurogenically inhibited. These studies were extended by van Tienhoven, Nalbandov & Norton (1954). It is tempting to suppose that ovulation is controlled by such an inhibitory mechanism in combination with a positive stimulation arising probably from the ruptured

follicle of the previous ovum. A hypothesis of this type has been very carefully discussed by Fraps (1955a)—a paper with an inappropriate title. (Bastian & Zarrow (1955) and Fraps (1955b) discuss the cycle of the hen in detail, but these papers are of less general interest). However, not only is such a system difficult to fit to the known timing of laying and ovulation in the hen (see Huston & Nalbandov, 1953) but also Nalbandov (1959) provides evidence that the inhibitory effect arises only from a certain region of the oviduct through which the egg passes at a time when it could not serve such a function in relation to the subsequent ovulation, and Nalbandov therefore proposes an entirely different function for the inhibitory effect.

Cole & Hutt (1953) studied hens which while ovulating normally did not lay because the yolks apparently did not enter the oviduct. Although these birds never had shelled eggs in the uterus ready to lay, they went to nest in much the same way as normal birds, suggesting that going to nest is probably induced by the hormones which bring about ovulation rather than by the presence of a complete egg. More work needs to be done on the physiology and behaviour of egg-laying.

Another problem which requires attention is on the control of the end of laying (viz. the completion of the clutch) and its possible relationship to the onset of incubation. The physiological control of clutch size, which may vary with season as well as with species, is of considerable interest, especially in view of the evolutionary aspects discussed by Lack (1947-8). For a review on clutch size see Davis (1955a). The question of determinate and indeterminate laying needs to be carefully considered before further hypotheses can be advanced. A bird described as a determinate layer lays a given number of eggs and then stops, regardless of interference with the number of eggs in the nest, while an indeterminate layer is influenced by interference with the number of eggs and is supposed to continue laying at the normal interval until the normal clutch is restored if eggs have been removed. The evidence for this traditional distinction has been reviewed by Davis (1955b) who concludes that it is insufficient and that there is no clear proof that any species is indeterminate if this evidence is examined by stringent standards. However, the evidence given by Paludan (1951) and Weidmann (1956) on three species of gull adequately shows them to be indeterminate layers, so that the distinct-

tion must be maintained.

The most obvious hypothesis for the control of the end of laying is that it is determined by a rise in the level of prolactin or progesterone. Although small doses of progesterone induce ovulation, larger doses inhibit laying in the hen Riddle & Bates, 1939), pigeon (Dunham & Riddle, 1942) and turkey (van Tienhoven, 1958). Prolactin also stops egg production comparably according to Riddle & Bates (1939), but Juhn & Harris (1956) provide some contradictory evidence although an antigonad effect of prolactin is well established (see p. 161). Again it must be remembered that prolactin may influence progesterone secretion. In birds where laying is determinate, the end of laying could be predetermined in that only the given number of follicles begin to mature at any one time or it could depend either upon an accumulation of progesterone resulting from a given number of ovulations, or upon the prolactin level reached during this period, without any dependence upon external stimuli. Where laying is indeterminate any such 'automatic' mechanism must be excluded, for environmental stimuli obviously come into play. As part of the evidence for indeterminacy in gulls, Paludan (1951) and Weidmann (1956) showed that more than the normal clutch number of follicles began maturation. If the gulls were given the opportunity to incubate the first eggs of the clutch, the supernumerary follicles were not developed further. I have elsewhere (Eisner, 1958) discussed these findings and suggested that the onset of incubation leads to increased prolactin secretion which then leads to the termination of laying. It is essential to such a hypothesis that there should be evidence of a rise in prolactin secretion at this time.

There appears to be quite a high level of oestrogen and androgen secretion in the female bird at the time of laying (see pp. 158-159). In rats and other small mammals, oestrogen or androgen treatment causes great increases in the pituitary prolactin content, oestrogen being especially effective (Meites & Turner, 1947). This has been directly investigated in birds only in the pigeon (Meites & Turner, 1947) and dove (Lehrman, unpublished) and such an effect was not shown; however, it would be unwise to generalize from these results as the pigeons are exceptional in possessing milk-producing crops whose activity is controlled by prolactin, so that the whole physiological system concerned with prolactin may also be a typical in their case. This objection can be substantiated because Bailey's experi-

ments (1952) on passerine birds strongly suggest that oestrogen does stimulate prolactin secretion. Stimulation of prolactin secretion by increase in oestrogen levels provides one possible mechanism which may be relevant in the case of determinate layers. In the case of indeterminate layers a mechanism involving response to environmental stimuli must be postulated and as there is evidence that prolactin secretion can be thus affected (see Eisner, 1958, and below; also Grosvenor & Turner, 1957) this seems a likely intermediary. Whatever the mechanism, there certainly are indications that the level of prolactin secretion increases during the laying period, although the peak is not reached until some time afterwards. Thus, Legait & Legait (1954) found that the number of mitoses in the anterior pituitary of the hen increased with the onset of laying and further with the beginning of incubation, and at this time were mostly in the acidophils which are recognized as the source of prolactin (see Martinet, 1952; Pearse, 1952). Further, Legait (1955) studied the neurosecretory nuclei of the hypothalamus in the hen and found that the activity increased during laying and early incubation, and then regressed: these changes were compared with the response to various treatments and it was found that injection of prolactin in small doses increased activity similarly to the increase found at the beginning of incubation, while larger doses suppressed activity comparably to the state normally occurring at the end of incubation. Lahr & Riddle (1938), from examination of the rate of mitosis in the crops of pigeons, give evidence of a rapid rise in prolactin between the laying of the first and second eggs, again corresponding with the beginning of incubation. Observations on the development of brood patch oedema during the laying period of several passerines (Bailey, 1952) also suggest a rise in the secretion of prolactin at this time.

The other essential to the confirmation of a hypothesis that prolactin controls the termination of laying could be to establish that inhibition of ovulation could result from prolactin doses compatible with the levels occurring normally in the bird at this period.

The Anti-gonad Effect of Prolactin

An anti-gonad effect of prolactin has been well established in birds (Bates, Riddle & Lahr, 1937; Bailey, 1950; Lofts & Marshall, 1956 & 1958). In the hen (Bates, Lahr & Riddle, 1935), the female pigeon (Bates, Riddle & Lahr, 1937) experi-

and female White-crowned Sparrow (Bailey, 1950) treatment with prolactin inhibited the growth of ovarian follicles. Prolactin also causes regression of the testis of male birds in a wide range of species (see Lofts & Marshall, 1956). This effect has usually been discussed in terms of an inhibition of FSH secretion, but investigations of the mechanism (see Lofts & Marshall, 1958) do not yet provide conclusive evidence, although it certainly appears that this is no direct effect of prolactin upon the testis. However, inhibition of FSH alone would not account for all the relevant effects of prolactin treatment. There is evidence of an inhibition of LH by prolactin in the apparent suppression of androgen secretion in both males and females: androgen secretion by both testis and ovary depends on LH. Collias (1950, p. 294) states that there is shrinkage of the comb in broody hens; Noble & Wurm (1940) note that the courtship colours, which depend upon androgen, fade during incubation in the Night Heron: there is evidence (see below et seq.) of a high level of prolactin secretion during incubation. Bailey (1950) found that whereas male White-crowned Sparrows stimulated by light sing frequently, birds simultaneously treated with prolactin do not. Ring Doves treated with prolactin ceased to behave sexually (Lehrman, 1955). In any satisfactory hypothesis of the anti-gonad effect it must therefore be postulated that prolactin inhibits the secretion of LH as well as that of FSH.

The Development of the Brood Patches and the Beginning of Incubation

There is unfortunately too little observational evidence on when exactly the brood patches begin to develop. There is clear evidence only for several species of female passerine that development begins several days before the first egg is laid (Nice, 1937; Bailey, 1952; Brackbill, 1958). The first apparent change is a loss of feathers; this is followed by a gradual vascularization which is complete soon after the last egg is laid; an oedema develops more slowly than the vascularization and is not complete till later (Bailey, 1952). These changes could be experimentally reproduced in intact birds by oestrogen treatment, except that they then occurred in a different order (Bailey, 1952)—a difference which Bailey does not discuss, although it requires explanation. In hypophysectomized birds oestrogen alone induced only vascularization of the areas, and subsequent prolactin injection was necessary to produce loss of feathers and

oedema; prolactin without oestrogen was ineffective (Bailey, 1952). Legait (1955) noted that prolactin injection of (apparently laying) hens induced brood patch development. Bailey (1952) found that brood patches could not be induced by androgen treatment in the passerine species used, but oestrogen treatment of the males which do not normally develop patches induced complete development of them. Bailey therefore raised the question of how brood patches were developed in the male non-passerines which had them, and suggested that androgen may be effective in these species. It must not, however, be forgotten that male birds secrete oestrogen as well as androgen.

During the laying period, mating and nest-building behaviour decline and incubation behaviour may begin. The time relationships are very variable from species to species and the changes are probably usually gradual. In by far the majority of species the female bird incubates and looks after the young. Males of some species participate neither in incubation nor in raising the young, and these do not concern us here. In other species the male does not incubate but remains with the female and feeds her and/or the chicks. In other species still the male shares incubation and care of the young more or less equally with the female.

A question of great interest is how far incubation behaviour depends upon the development of brood patches. Certainly, it also depends on the presence of particular stimulus situations (nest and eggs) and may be induced by the presentation of such stimuli at abnormal times (see Emlen, 1941; Poulsen, 1953). In non-passerine birds, except in the very few species in which the eggs are not incubated in the usual way (see Tucker, 1943), the development of brood patches appears to correspond precisely with participation in incubation (see Bailey, 1952; 1955). Unfortunately, this generalization appears not to hold in the passerines, as Skutch (1957) has pointed out. Although there are records of brood patches in male Tyrannid flycatchers (Davis, 1945; Parkes, 1953), there seems to be only one reliable record of an incubation patch in a male of the *Passeres*: in the Clark Nutcracker, a species in which the male does incubate (Mewaldt, 1952; 1956). While there are few families within the *Passeres* in which the males of most species share in incubation (*Prionopidae, Zosteropidae, Estrildidae*), there are some species within many families where the male does regularly participate (e.g.

Kluijver, 1933; Brown, 1946; Moreau, 1949; Crowe, 1955; Morel & Bourlière, 1956; see further Ryves, 1943; Kendeigh, 1952; Skutch, 1957). The evidence for male incubation among passerines is now altogether reliable in many cases, so that the cautionary suggestions of Ryves (1943) and Bailey (1952) are no longer valid objections. It is now necessary that more passerine species in which the male is known to incubate be examined at the appropriate time for the presence of brood patches. Statements such as that of Parkes (1953) that breeding males of 41 unspecified species of *Passeres* were examined without a brood patch being found are of limited value. There is a considerable amount of reliable evidence that in many passerine species males may truly incubate without developing brood patches. Erickson (1938) found that in the Wren-tit (*Chamaea*) the male shares incubation equally with the female by day, and both make identical settling movements, but Miller (1944) reports that the male Wren-tit has a bare brooding area during incubation but that it is not highly vascular as in the female. Miller (1944) notes further that he has examined incubating males of other passerines and found little or no development of a breast area free of down. Addicott (1938) observed that in the Bush-tit (*Psaltriparus*) both birds incubate equally by day and stay together on the nest at night, yet the male does not develop a brood patch; Bailey (1952) also did not find brood patches in males of this species. In the Black-headed Grosbeak the male incubates for 40 per cent. of the time by day (Weston, 1947) but again Bailey (1952) found no male incubation patch in this species. The best example is undoubtedly the study by Petersen (1955) of the Bank Swallow, in which the male and female share time on the nest about equally, and the male may even be on the nest alone at night. A thermometer introduced among the eggs showed marked rises in temperature when the male was on, but brood patches were not found in the male birds. There is evidence in other passerine species that the males can warm the eggs. Smith (1950) found that the male Yellow Wagtail takes an appreciable part in incubation and makes proper settling movements and that when he leaves the nest the eggs are warm; unfortunately Smith did not examine the birds closely to see whether a patch was present, but he saw the skin of the breast was bare of feathers. A most extraordinary instance is described by Kuerzi (1941): in the Tree Swallow normally only the female incubates,

but in one case the female died after three days' incubation and the male took over, hatching and rearing the young successfully; again, however, the bird was not examined for the presence of a brood patch.

It seems, thus, that male passerines of certain species do show true incubation behaviour and can warm the eggs although they may not possess fully developed incubation patches. In view of this information on other species, I was very interested to see whether in my Bengalese Finches, where there appears to be no sex difference in incubation and brooding behaviour, brood patches were developed. I examined males and females at various points of the incubation and parental periods and could find no difference in the condition of the ventral surface between male and female. Neither sex showed any development which could truly be called a brood patch, and parental birds were not obviously different from mature birds which were not nesting. The ventral surface of mature birds is at all times free of down feathers, and in the nesting birds I could see no suggestion of vascularization or oedema, the ventral skin of incubating birds being quite normally transparent and delicate. Thus neither male nor female of the Bengalese Finch, although it incubates very intensively, develops a brood patch at all.

It is apparent therefore that incubation behaviour in passerines is not necessarily correlated with the possession of a brood patch, but in non-passerines it may be. I am aware of no experiments which have attempted to investigate this point directly, and there is far too little observational information on how the development of the brood patch normally corresponds in time with the beginning of incubation. In the female passerines on which information is available (Nice, 1937; Bailey, 1952; Brackbill, 1958) the patches begin to develop before the eggs are laid and the period of greatest change probably corresponds with the time at which incubation behaviour is developing: the development of the patches may be ascribed simply to the hormonal changes which are necessarily occurring at that time. Unfortunately there is no information on the exact time at which males which incubate develop brood patches, in those species where this occurs. The question of interest is how far the development of the patches and the beginning of incubation behaviour, especially in the male, depend on previous hormone changes, and how far they are influenced by the stimulus situation of eggs in the nest.

Before attempting to elucidate the normal sequence of events, it is necessary to discuss the results of experiments on the induction of incubation behaviour. There is considerable confusion in some of the literature of hormone experiments because certain clear distinctions have not been emphasized. Responses both to eggs and chicks have been lumped together as 'broody behaviour' and, as a result, certain quite characteristic failures have seemed anomalous rather than significant. The periods of incubation of eggs and of care of young are, one would have thought, quite obviously distinct behaviourally (as was discussed by Pearl, 1914) and it has now been shown (Saeki & Tanabe, 1955) that they correspond with distinct physiological states of the parent. It is therefore necessary to emphasize that the indiscriminate use of the word 'broody' is most undesirable.

Incubation of eggs is undoubtedly more difficult to induce in fowls than is the behaviour associated with the care of chicks. Hens become broody comparatively easily as a result of exposure to chicks, but respond much less readily to accumulations of eggs (Collias, 1946). Prolactin treatment is effective in inducing incubation only with specific limitations. Neither cocks (Nalbandov, Hochhauser & Dugas, 1945) nor capons (Saeki & Tanabe, 1955) can be induced to incubate eggs by prolactin treatment, although they will take care of chicks. There are known genetic factors in whose absence broodiness cannot be induced and, given the necessary genetic constitution, incubation of eggs follows prolactin treatment alone only if the bird is in active egg production at the time (Byerly & Burrows, 1936; Riddle & Bates, 1939). Non-laying hens in moult can, however, be induced to incubate eggs if prolactin treatment is combined with cooping in darkness and warmth although such cooping alone is ineffective (Saeki & Tanabe, 1955).

On the basis of the earlier evidence, it seemed likely that prolactin was effective in inducing incubation only in circumstances where it would also induce brood patch development. Bailey (1952) found that both oestrogen and prolactin were necessary for the full development of the patch, and a sufficient level of oestrogen would probably not be present in cocks, capons or non-laying hens. This case has been argued by Lehrman (1955). However, there is contradictory evidence, although of a rather fragmentary nature, and also it is difficult to see why

the addition of cooping to prolactin treatment should have proved effective in non-laying hens (Saeki & Tanabe, 1955), for sex hormone secretion tends to be inhibited by darkness. Saeki & Tanabe also treated a capon with oestrogen as well as prolactin without inducing incubation, although this treatment should have caused brood patch formation if Bailey's evidence (1952) is at all relevant to the fowl (Legait, 1955, noted that prolactin treatment of apparently laying hens did produce development of brood patches, which is consistent with Bailey's results). Of course evidence on a single bird is not of sufficient weight in itself, especially as the bird was not examined for the presence of a brood patch. Different evidence against the argument that brood patch development is essential for the induction of incubation comes from cases in which incubation behaviour was shown although brood patch development probably did not take place. The situation in passerine birds has already been discussed, but there is also relevant information on pigeons. Collias (1946) gave oestrogen injections to a hypophysectomized male, by which he was induced to pair with a female: this male then shared normally in incubation but did not produce crop-milk, confirming the absence of prolactin. There is no comment on the condition of the brood patch: vascularization might have been shown but development would surely not have been complete. Male pigeons castrated while their mates are laying could probably be expected to develop brood patches normally, and they have been found to incubate (Riddle & Dykshorn, 1932; Patel, 1936). Such paired castrated males may remain paired after the first cycle is over, though they no longer behave sexually. Kaufman & Dabrowska (1931), Kaufman (1932) and Patel (1936) report that such birds may incubate normally even after considerable periods of time since their operation although again they do not produce crop-milk; here also there is no comment on the state of the brood patches, which presumably were not developed.

Lehrman (1955; 1956) postulates that a bird with developed brood patches finds relief from peripheral irritation by sitting on eggs, and therefore incubates. Before such a hypothesis can be seriously considered, it must be established how far incubation behaviour in fact depends upon the presence of brood patches. Clearly, the whole matter is one on which further experiment is required.

Whether or not it is supposed that incubation behaviour usually depends upon the presence of brood patches, it is necessary to have satisfactory evidence on what hormone treatments can induce incubation behaviour and on how these treatments relate to the physiological situation at the normal start of incubation and at times when incubation is induced by supplying appropriate stimuli artificially. Prolactin has been supposed to be the hormone controlling incubation and parental behaviour because prolactin treatment can sometimes induce such behaviour, and because there is evidence of a high rate of prolactin secretion during the incubation period (see later). In fact, until the experiments of Lehrman which are not yet published, the hen was the only bird on which the effect of prolactin upon incubation behaviour had been tested (see p. 163). Although so much work had been done on prolactin using pigeons, this particular experiment had not been tried. Riddle & Lahr (1944) induced incubation of eggs in Ring Doves by administration of progesterone, testosterone and desoxycorticosterone and claimed that these treatments were effective because they stimulated prolactin secretion, but this interpretation is certainly not valid (see Lehrman, 1958b; Eisner, 1958). Lehrman has recently made a most careful study of the problem of the onset of incubation in Ring Doves (1958 a, b & unpublished) and suggests that the hormone most directly concerned with the induction of incubation behaviour in doves is progesterone, there being no evidence that prolactin is involved at this time. The evidence suggests instead that incubation, once established, then stimulates prolactin secretion. It would, however, be unwise to generalize to other species from these findings on the doves before further evidence is available. The doves and pigeons may be atypical in their hormone physiology (see p. 160). Also Lehrman's evidence suggests that the progesterone secretion which prepares the doves for incubation is stimulated by the behaviour of nest-building. There are, however, many species in which a bird may build but does not then incubate (e.g. male Wren, many ploceine males) and also some in which a bird incubates without having previously participated in nest-building. Thus in *Quelea quelea* both male and female incubate, but the nest is built entirely by the male (Morel & Bourlière, 1956). In the Reed-warbler, *Acrocephalus scirpaceus*, the unpaired males build "cock's nests," but the true nest is built entirely by the female, and yet the male shares in incubation (Brown,

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1946). In the Yellow Wagtail and the Rose-breasted and Black-headed Grosbeaks, the males contribute appreciably to incubation without having built at all (Smith, 1950; Ivor, 1944; Weston, 1947). There are of course also birds such as the Nightjars in which no nest is built (Witherby, *et al.*, 1943). Clearly, incubation does not necessarily follow upon nest-building in any one individual although, as Lehrman (1959) suggests, building by one sex may have a stimulatory effect upon the other. It may also not be assumed that the onset of incubation is mediated by progesterone in species other than the doves, since Riddle, Bates & Lahr (1935) found that progesterone treatment did not induce hens to incubate although prolactin treatment did.

However important the internal, hormonal, situation may be, the importance of environmental stimuli at this time must not be underestimated. Incubation of eggs can often be induced by presenting a nest and/or eggs considerably before the eggs would normally be laid (see Emlen, 1941; Poulsen, 1953). For instance, Noble, Wurm & Schmidt (1938) found that the male Black-crowned Night Heron 'is greatly stimulated by the sight of an egg in his nest even ... before his mate has laid. He at once begins to brood ...' There seems to be no evidence suggesting that incubation can be so induced in birds not in reproductive condition, but it seems unlikely that either progesterone or prolactin is necessarily present in significant quantity before such experiments are successful. In the normal cycle, incubation usually develops rather gradually and probably stimuli from the eggs lead to hormonal changes which in their turn intensify the behaviour.

In the female the normal onset of incubation, especially if laying is determinate, possibly depends upon hormonal changes associated with the laying of the clutch. In the male such a mechanism cannot be postulated, and it is therefore necessary to determine how far the development of the brood patches and the response to eggs depend on hormone changes taking place during the preceding phase, and how far they are influenced by the stimulus situation. In many cases there is no suggestion that there might be relevant hormone changes in the male before the eggs have appeared. The male of most pigeon species does not begin incubation until after the first egg is laid (at which time the female begins) and sometimes not until after the laying of the second egg

(Whitman, 1919, p. 54). However, Whitman (1919, p. 53) says that the Passenger Pigeon began to incubate a day or two before laying began, the male taking his turn on the nest 'just as if the eggs were there.' Even if this was exceptional among pigeons, it is of great interest. Less obviously relevant observations may also be suggestive. I found in the Bengalese Finch, which normally has very dry faeces, that the beginning of laying could often be predicted from a softening of the faeces which occurred in both male and female some two days previously and persisted throughout the incubation period. This suggests that some similar change is taking place in both birds, and it may be a hormonal change: indeed, this perhaps suggests that the beginning of incubation in this species may not be associated with a rise in progesterone secretion as Riddle & Lahr (1944) reported that doves treated with progesterone developed intestinal stasis and, comparably, constipation is frequent during human pregnancy (Browne & Browne, 1955). There are often changes in other behaviour associated with incubation, such as a reduction in sex behaviour and song, and it might be useful to have evidence on the exact timing of these changes and in particular to know whether they occur before incubation begins. In the Starling the male stops singing when incubation begins, the male sharing incubation equally with the female; there is no suggestion of a change before there are eggs in the nest (Kluijver, 1933). The studies of changes in the pituitary and gonads are not usually sufficiently closely graded in time to provide useful information on this point. Testis size (as well as of course ovary size) is usually maximal at about the time the eggs are laid, this size sometimes being reached considerably earlier (Marshall, 1949; Johnston, 1956) and sometimes only just before or during the laying period (Petersen, 1955). As in the three species of the above studies the males regularly incubate, the exact timing of testis growth is probably of little relevance to the normal onset of incubation. There is evidence in females of several species and in the male pigeon of a rise in prolactin secretion during the laying period (see p. 160-161) but this seems to occur together with the beginning of incubation, rather than before it.

The Incubation Period

Whatever the causes of the onset of incubation, there seems to be a good deal of evidence that prolactin is associated with the incubation

period. There is both direct (assay) and indirect (cytological) evidence of a high rate of prolactin secretion during incubation in male and female pigeons (Schooley & Riddle, 1938; Lahr & Riddle, 1938) and in hens (Saeki & Tanabe, 1955; Legait, 1955; Nakajo & Tanaka, 1956), and assay evidence of this in California Gulls of both sexes (Bailey, 1952) and in pheasants (Breitenbach & Meyer, 1959). There is also a good deal of indirect evidence in the regression of gonads and suppression of sex behaviour during incubation, if this is taken as resulting from the anti-gonad effect of prolactin.

The male domestic pigeon shows constant spermatogenesis and sex drive when it is incubating or feeding young: there is a drop in testis weight with the beginning of incubation, and this change correlates with changes in pituitary cytology and in the crop which suggest prolactin secretion (Schooley, 1937). It is a frequent observation in ornithology that later in the breeding season it is only the unmated males of a particular species which sing (e.g. Burkitt, 1919; 1921; Kluijver, 1933). Burkitt, however, noticed that in the species which he studied this applied only to those in which the male showed parental behaviour, but did not apply to the Chiffchaff and the Wren where the male does not help in raising the young. Kluijver (1933) found that in the Starling the male incubated and that song was at its strongest during the laying period and decreased as soon as incubation began. In the Black-headed Grosbeak the male incubates regularly and although song does not cease altogether at this time it is much reduced (Weston, 1947). Similarly, I have found song to be extremely rare in incubating Bengalese Finches. In contrast in varous species where the male does not incubate he continues to sing (Blanchard, 1941; Kuusisto, 1941; Lawrence, 1953). There thus appears to be some correlation between absence of singing during the incubation period and the participation of the male in incubation, but unfortunately there are exceptions which suggest that this evidence must be taken with caution. The Great Reed-Warbler in Germany does not share in incubation but his song ceases almost completely while the female incubates (Kluijver, 1955) and similarly the song of the male Red-breasted Flycatcher ceases as soon as his mate starts consistent incubation although he does not himself incubate (Weber, 1958). In contrast, the male Wren-tit shares in incubation equally with the female but none the less sings regularly and frequently during his periods off

the nest (Erickson, 1938). Also, song may be reduced at other periods of the reproductive cycle when it is unlikely to be caused by increased prolactin secretion. The song of a territorial male may be markedly reduced when a female enters his territory and pair formation begins, but then increases again later (Tinbergen, 1939; Nice, 1943; Lawrence, 1953). In the Rufous-sided Towhee the song of the male, which does not incubate, decreases after the young hatch but this is not correlated with any regression of the gonads, for testis regression does not begin until about a month later (Davis, 1958). Therefore, although the cessation of song during incubation may depend upon an increased rate of prolactin secretion and a consequent reduction of androgen output, it would be unwise to assume this without more direct evidence. Changes in secondary sexual characters are probably more reliable indicators of decreased sex hormone production. Collias (1950; p. 294) states that the comb, whose size certainly depends upon androgen secretion, shrinks in broody hens, and that the ovary is regressed and copulation does not take place during broodiness: this corresponds with ample evidence of a high rate of prolactin secretion. The courtship colours of the Black-crowned Night Heron, which also depend upon androgen, fade during the incubation period (Noble & Wurm, 1940). It would be valuable to have more information of this type, which should he comparatively easy to obtain, especially as it is known how such things as beak colour are controlled in a variety of species (see Domm, 1939).

Finally, the available evidence on the state of the gonads during incubation is of considerable importance. In both male and female pigeons (Schooley, 1937) and in hens (Collias, 1950) the gonads are definitely regressed at this time, corresponding with the more direct evidence of prolactin secretion. In the California Gull there is also evidence of prolactin secretion during incubation (Bailey, 1952), and the testes of the males begin to regress at about the time the eggs are laid and incubation begins and have decreased considerably in size by the time the eggs hatch (Johnston, 1956). In the Fulmar also testis regression begins at about the time when incubation is beginning, and the testes are very much smaller and in a state of marked reorganization by the time of hatching (Marshall, 1949). Thus the available evidence on non-passerines which incubate their eggs is consistent with there being a high rate of pro-

lactin secretion at this time. The evidence on passerines however, is again contradictory. In the female House Sparrow the ovary is regressed during incubation, although not as much as out of season (Vaugien, 1955), and in the Bank Swallow also the ovary and oviduct regress rapidly at the beginning of incubation (Petersen, 1955), but incubating female Rooks have ovaries with many large follicles and the ovaries and oviducts do not appear to regress until after the young have hatched (Marshall & Coombs, 1957). The available evidence on male passerines does not at all fit the suggestion that incubation corresponds with a high rate of prolactin secretion, if it is accepted that prolactin generally has an anti-gonad effect in birds. In *Quelea quelea*, in which the male incubates, the postnuptial steatogenesis (collapse and reorganization) of the testis is delayed until well after the young have hatched (Disney & Marshall, 1956). The male incubates in the Bank Swallow, and again the testes remain at maximum size during the incubation period and begin to regress and reorganize only at about the time of hatching (Petersen, 1955). In contrast, in the male Rook, which does not incubate, testis regression begins soon after the eggs are laid and is already quite marked by the time of hatching (Marshall & Coombs, 1957). These results together with the absence of brood patches in incubating male passerines probably suggest that they do not secrete prolactin during incubation: more direct evidence on the matter would be very desirable. Collias's observation (1946) of a hypophysectomized pigeon which incubated normally is clear evidence that this behaviour is not necessarily dependent upon the secretion of prolactin.

Once incubation has begun, it must then be maintained for the period needed to hatch the eggs. This maintenance clearly involves the presence of the eggs: if the eggs are removed most birds will not continue to show incubation behaviour for the normal length of time, though they may continue to sit on the nest for a few hours or days. On the other hand, some birds will sit for exceedingly long periods on infertile eggs or dummies (Emlen, 1941; Poulsen, 1953; Weidmann, 1956). Saeki & Tanabe (1955) and Patel (1936) showed that in such circumstances prolactin secretion was maintained in, respectively, the hen and the pigeon, which is very good evidence for the environmental stimulation of prolactin secretion. Patel (1936) showed further that tactile stimulation from the eggs was not essential for the maintenance of pro-

lactin secretion. If the male of an incubating pair of pigeons was separated from the female and the nest by a transparent screen, the female incubated almost continuously. The male, while thus prevented from brooding, became restless whenever she left the next and quietened when she returned. At the same time his crop continued to develop normally and he was capable of feeding the squabs at the due time, showing that prolactin was secreted. If, however, the male was taken out of sight of the female, his crop regressed and after two days he showed normal non-broody behaviour. This shows that the behaviour of the mate as well as the presence of the nest and eggs can be important in the maintenance of prolactin secretion.

In the hen, pigeon, California Gull and pheasant, at least, there is adequate evidence that prolactin is present in considerable quantity during the incubation period. Further, the evidence suggests that the act of incubation or stimuli associated with it promotes the secretion of prolactin. This is shown by Patel (1936) in pigeons and Saeki & Tanabe (1955) in hens, by experiments in which incubation was artificially prolonged, and suggested by the experiments of Poulsen (1953) on pigeons and Weidmann (1956) on gulls in which the normal laying of the clutch was prevented by prematurely giving the birds artificial eggs which they then incubated. This probably is also the correct interpretation of Riddle & Lahr's experiments (1944) (see p. 164-165). If prolactin is present but does not control incubation behaviour, one must suppose that it has some other function or functions. Its probable function in the control of brood patches has already been discussed. In the pigeons it is of obvious importance in the control of crop-milk production: corresponding with the development of the crop during incubation there is a considerable increase in liver size (Schooley, 1937), and this and various metabolic effects have been found to result from prolactin treatment (see Riddle & Bates, 1939). In the hen there certainly are marked metabolic changes associated with broodiness, which are evidenced in decreased food-consumption (Hellwald, 1931; cited by Wood-Gush, 1955). Prolactin may well have important metabolic effects which are necessary for incubation in birds other than the pigeons, where such effects may be interpreted as essential for crop-milk production. There is also the anti-gonad effect which may be very relevant to incubation and parental behaviour: this will be discussed further

below.

The Period of Care of the Young

After incubation comes the change-over from behaviour appropriate to the eggs to that involved in the care of the young. The main patterns shown towards the young differ with different families of birds. In passerines they are brooding (at least for the first few days), feeding and removal of the faeces, in many other birds such as the pigeons and the gulls they include brooding and feeding, in the fowl there is brooding and calling to food (viz. clucking), various species such as the ducks and geese lead their young, and so on. In the passerines, near-passerines and other birds in which the young hatch in an undeveloped state and remain in the nest, brooding of them may probably be considered an extension of egg-incubation. In species where the male does not incubate he will not brood chicks even though he feeds them. Where the chicks are precocious, as in the gulls and the fowl, brooding behaviour may be rather different from incubation and different postures may be used. Thus a hen tends to brood her chicks under the wings (see e.g. Ramsay, 1953). Feeding, removal of faeces, leading, etc., are clearly something new and distinct.

For convenience I will use the term 'parental behaviour' here to refer to all activities used in the care of the young except incubation-type brooding. The first evident point is that such parental behaviour is very greatly dependent upon the stimulus situation. It is much easier to induce, either with or without hormone treatment, than incubation. Excepting the brooding of nest-bound young and the rather special case of the pigeons where crop development is involved, feeding and general care of young birds do not appear to involve any specially developed structures without which such behaviour could not be shown. This probably is a large part of the explanation of the different ease of induction of parental and incubation responses. In mammals, similarly, general parental behaviour can often be quite easily induced, independently of lactation (see e.g. Leblond & Nelson, 1937). The variety of circumstances in which such parental behaviour can be induced suggests strongly that there is no very precise hormonal condition which is a necessary pre-requisite, and this suggestion is confirmed by some experimental results. In particular there is little suggestion that prolactin is always directly necessary for parental behaviour, although it may facilitate its induction.

Emlen (1941) found in the Tri-coloured Redwing that young introduced into the nest before laying began were either neglected or caused desertion, if they were introduced during the laying period the males (which do not incubate) accepted and fed them but the females would not respond until after they had finished laying, when any young in the nest always took precedence over the eggs. Although this shows that there are circumstances in which response to young is inhibited, it also shows that young may be accepted appreciably before they would normally appear. Similarly Kinsey (1935) found that a pair of Black Phoebes with fresh eggs accepted some newly hatched nestlings, the male feeding them very promptly, while another pair accepted nearly fledged young instead of slightly incubated eggs. Goethe (1953) gave pipped eggs to Herring Gulls: five pairs with clutches at least 12 days old accepted these 'premature' young, but a sixth pair with a fresh or perhaps even incomplete clutch did not.

In domestic fowl, laying hens can easily be made broody by keeping them with baby chicks but older chicks are less effective (Collias, 1946). Burrows & Byerly (1938) found that laying hens rapidly became broody towards chicks, and broodiness could also be induced in non-laying hens and immature pullets, though less easily, if chicks were given in certain environmental circumstances (darkness, warmth). (N.B. Such conditions will not induce incubation of eggs by non-laying birds, see p. 163). Pearl (1914) also reports that non-layers may become broody towards chicks. However, Ramsay (1953) found that the broody behaviour induced towards chicks in non-breeding hens was not complete. Capons which are cooped with chicks will become broody towards them (Goodale, 1916; Saeki & Tanabe, 1955) and Pearl (1914) says that this was a commercial practice. Nalbandov, Hochhauser & Dugas (1945) found that normal males would always neglect or attack chicks placed with them, but Saeki & Tanabe (1955) obtained broodiness in 2 of 6 cocks which were cooped with chicks, without hormone treatment (this may be partly a racial difference, cf. Nalbandov & Card, 1945).

Incubation in hens is associated with a high prolactin potency of the pituitary (p. 165), and because in much of the literature incubation and broodiness towards chicks were not distinguished it was supposed that the care of chicks

must also correlate with high prolactin secretion. Saeki & Tanabe (1955) have shown that this is not the case: they found that the prolactin potency of the hen pituitary fell after the eggs hatched to the level found in a normal laying hen, this level being reached probably about ten days after hatching although the chicks are cared for much longer. These results fit with several findings that after broodiness towards chicks has been induced there is no increase in pituitary prolactin (Burrows & Byerly, 1938 on hens; Saeki & Tanabe, 1955 on cocks and pullets) and that broodiness may continue after the prolactin potency has been reduced by electrical treatment (Nakajo & Tanaka, 1956). Also, Breitenbach & Meyer (1959) have found in the pheasant that the pituitary prolactin level rises only until mid-incubation and then begins to fall steadily, reaching a level comparable to that of laying birds within a few days after the chicks hatch.

In pigeons during the first part of the parental period there is certainly a high rate of prolactin secretion which is associated with the production of crop-milk. Lehrman's (1955) experiments showed that when Ring Doves were induced to accept young by prolactin treatment, this was, at least largely, due to the effect of prolactin on the crop, rather than to any more direct effect upon the central nervous system. However, mated castrate pigeons whose crops do not produce milk will care for young which they feed on seed (Kaufman & Dabrowska, 1931; Kaufman, 1932; Patel, 1936), and in normal birds the secretion of prolactin and the production of crop-milk do not persist throughout the parental period (Schooley, 1937) and thus during the later part of the period the squabs are cared for although prolactin is not secreted in large quantity: after the first few days the squabs are fed increasingly on seed (Patel, 1936). Thus even in the pigeon parental care is not necessarily dependent upon prolactin secretion. Further, Patel (1936) found that while crop activity (and therefore also prolactin secretion) can be maintained by maintaining incubation, the presence of squabs has no such effect: crop-milk production could not be maintained by constantly replacing the growing young with newly hatched ones, suggesting that care of the young does not stimulate prolactin secretion in the way that incubation appears to do.

Thus in the fowl, pheasant and pigeon there is evidence that care of young does not necessarily depend upon a high rate of prolactin secretion. A decline in prolactin secretion after the young

hatch is also indicated in some female passerines in which Bailey (1952) found that the brood patch begins to regress a few days after hatching. Also, birds may take care of young at times when there is no reason to expect a high prolactin potency, as for instance the male passerines which do not share in incubation but normally attend to their young. There are also the instances in which birds other than the parents may help at the nest without being themselves in the appropriate phase of the breeding cycle, especially the cases in which juveniles or immatures assist in feeding young (Skutch, 1935; White, 1941; Dexter, 1952).

Similarly, in mammals there is good evidence that parental care, apart of course from lactation, is not necessarily dependent upon a high level of prolactin secretion. Hypophysectomy of gravid rats tends to disturb parturition seriously, but those animals which survive this then show apparently normal parental behaviour although they do not lactate (Obias, 1957). Hypophysectomized mice respond to new-born young, and such responses can even be obtained from mice (of either sex) which are operated pre-pubertally and have therefore had no experience of their own young (Leblond & Nelson, 1937). Parental responses can be obtained from intact or castrated male mice and from virgin females without hormone treatment, although a 'sensitization period' of up to four days may be needed (Leblond, 1940). (N.B. There are often comparable delay periods when broodiness is induced in domestic fowl by the presentation of chicks, e.g. Saeki & Tanabe, 1955). In the vole, *Microtus arvalis*, the retrieving response may be shown by young 11-15 days old which are themselves only just mobile (Frank, 1952).

It is therefore evident that parental behaviour does not necessarily depend upon prolactin. However, this is not to say that prolactin is without effect upon parental behaviour, apart from its control of milk production in pigeons and mammals. In the domestic fowl, there appear to have been no experiments on whether prolactin facilitates the response to chicks of adult hens, but Saeki & Tanabe (1955) found that immature pullets treated with prolactin responded to chicks no quicker than did controls. But prolactin treatment of cocks induced complete parental behaviour towards chicks, which did not occur in untreated cocks (Nalbandov, Hochhauser & Dugas, 1945). Mature rats may respond to pups without hormone treatment, and only animals which did not so

respond were used for experiment by Riddle, Lahr & Bates (1942): prolactin (and other treatments) did induce parental behaviour in such animals. Prolactin will also induce care of young in Jewel Fish (Noble, Kumpf & Billings, 1938).

Nalbandov, Hochhauser & Dugas (1945) have suggested that it may be the anti-gonad effect of prolactin, with its concomitant influences on sexual and aggressive behaviour, which is important in the induction of parental behaviour, rather than some more direct effect of prolactin. The same possibility is also suggested by experiments in which parental behaviour has been induced by substances other than prolactin. Noble, Kumpf & Billings (1938) found that not only prolactin or general pituitary extracts induced brooding behaviour in experienced Jewel Fish, but so also did corpus luteum extracts (=progesterone) and, to a lesser extent, thyroxine and phenol solution (the prolactin had been made up in phenol and thus the phenol was tried as a control, and found to have a slight effect). Riddle, Lahr & Bates (1942) found progesterone, desoxycorticosterone, thyroxine, LH and phenol effective in rats, and testosterone was effective in all except mature males. Riddle & Lahr (1944) induced incubation of eggs in doves with progesterone, testosterone and desoxycorticosterone, and Lehrman (1958b) has confirmed this effect of progesterone and shown (unpublished) that in this case prolactin is not effective except perhaps in very large doses. The question is whether such treatments are effective because (a) they suppress the gonads, or (b) they induce prolactin secretion, or (c) they operate in some other way.

Firstly, the case of the doves. Lehrman's work has eliminated possibility (b), and Lahr & Riddle (1944) provide the relevant evidence on (a). Lahr & Riddle found that desoxycorticosterone and progesterone caused loss of testis weight, and testosterone while not affecting testis weight caused atrophy of the Leydig cells which secrete sex hormone. Thus the agents which induced incubation do have an adverse effect upon the testis; the effect is not in all cases comparable with that of prolactin, which is further evidence against possibility (b), while these results are consistent with possibility (a). Lahr & Riddle (1944) also tested the effect of other agents on the testis of the dove, and in particular found that phenol caused serious damage to the Leydig cells. If these findings can be generalized to the Jewel Fish, they might explain Noble, Kumpf &

Billings's results (1938), but see further below. The findings on the rat are also mostly consistent with this interpretation, as Riddle, Lahr & Bates (1942) point out, except for the effect of LH which is known to stimulate androgen secretion.

Experiments on castrates are obviously relevant if it is suggested that these various hormones operate via suppression of the gonads. If the treatments operate only via suppression of the gonads, then (a) castrates should be more responsive to appropriate stimuli without treatment than are normals, and (b) hormone treatments should not affect the behaviour of castrates. If the anti-gonad effect is irrelevant, there should be no difference between normals and castrates in parental behaviour. If both suppression of the gonads and some other effect of the treatment are relevant, castrates should be more responsive than normals both with and without treatment.

The series of experiments on parental behaviour using castrates of the Jewel Fish (Hemichromis bimaculatus: Noble, Kumpf & Billings, 1938) is scarcely adequate. Noble et al. state that untreated fish do not respond to young except when they are nearing the appropriate stage of their own cycle and that castrates also are unresponsive; however, all fish used in experiments were pre-tested, only those which actually ate young given to them being used. It is stated that castrates required less prolactin and responded more frequently to prolactin treatment than did normals, but this statement is not supported by the data given: 17/20 normals and 14/16 castrates showed broodiness after treatment, which does not at all suggest any difference, and neither is the evidence on dosage clear. There was no difference between castrates and normals in response to progesterone which induced broodiness in all cases (9 normals and 4 castrates), or to phenol. It is evident from thse results that parental behaviour is facilitated in castrates by these treatments, which suggests that they do not operate solely by suppression of the gonads. Riddle, Lahr & Bates (1942) used adequate numbers of normal and castrated rats of both sexes, and presented their results clearly. They found that about 25 per cent. of normal rats of both sexes respond to pups without treatment, and those which do not may respond after treatment with prolactin, progesterone or some other agents. Slightly more castrates than normals respond to pups without previous treatment, and castrates also tend to be more re-

sponsive to certain treatments. The response to large prolactin doses is greater in castrates than in normals, especially in males; the response to LH is also greater in male castrates than in intact males, but there is no difference in response to LH between normal and spayed females; there was a greater response to testosterone in castrates of both sexes than in normals. However, there was no difference in response to desoxycorticosterone and to progesterone between normals and castrates. These results show that loss of the gonads does facilitate parental behaviour slightly, but that this certainly is not the sole explanation. The latter is suggested especially by the power of testosterone in inducing parental responses in castrates and by the pronounced effects of the other treatments upon them. There are unfortunately no comparable experiments on birds which have been castrated. While capons certainly become parental more easily than do intact cocks (see p. 168) no experiments on the effect of hormone treatments on the response of capons to chicks have been carried out. The anti-gonad effect is probably very important in the induction of parental behaviour in cocks by prolactin treatment: the cocks become broody gradually and the increasing parental behaviour corresponds with changes indicating androgen decrease, such as suppression of crowing and reduction of comb size (Nalbandov, Hochhauser & Dugas, 1945). In hens, however, lack of sexual condition does not facilitate broodiness: it is distinctly more difficult to make non-laying birds broody to chicks than laying birds. It seems probable that in birds suppression of the gonads may facilitate parental behaviour, but that this does not entirely explain the influence of hormone treatments.

There are two probable reasons for the importance of gonad suppression to parental behaviour. Firstly, birds in sexual condition may attack young or respond sexually towards them, which may be equally dangerous. This frequently happens when chicks are placed with cockerels (Nalbandov, Hochhauser & Dugas, 1945). Similarly, Lehrman (1955) found that untreated Ring Doves were very likely to kill squabs placed with them because they responded sexually and aggressively. Both in the cocks and the doves such behaviour was suppressed after prolactin treatment. Secondly, even if such responses are not shown it may be necessary to suppress sex hormone secretion as these hormones can have an inhibitory effect upon parental behaviour. Riddle, Lahr & Bates (1942)

found that while testosterone tended to facilitate parental behaviour in rats, rats never showed parental responses while receiving oestrogen injections, although they became very responsive as soon as treatment ended. (Oestrogen treatment induced mammary growth: when the treatment was ended lactation began. There is a large literature on the mechanism by which lactation is inhibited during pregnancy: this appears to involve a synergism of progesterone and oestrogen, although oestrogen alone can inhibit lactation. See Folley, 1956.). Weichert & Kerrigan (1942) gave oestrogen injections to rats daily from the day of parturition. On the 6th or 7th day the behaviour began to be abnormal: care of the young became sporadic and gradually less frequent, the pups were scattered and allowed to get cold and were not permitted to suckle normally. Broodiness in hens both to eggs and to chicks can be interrupted by treatment with oestrogen or androgen (Collias, 1940; Roux, 1947; Godfrey & Jaap, 1950). and simultaneous treatment with FSH or androgen prevents the induction of broodiness in cocks by prolactin treatment (Nalbandov, Hochhauser & Dugas, 1945). There is further evidence that high androgen secretion is antagonistic to parental behaviour in Ramsay's experiments (1953) in which dominant hens took longer to become broody towards chicks than did subordinates. However, although testosterone can prevent the establishment of incubation of eggs in turkeys, neither oestrogen nor androgen treatment will interrupt it (van Tienhoven, 1958). In pigeons also sex hormone treatment appears not to disturb incubation (Schooley, 1937; Collias, 1940). There is no evidence on whether sex hormone treatment would disturb established responses to young in turkeys and pigeons. There is ample evidence in wild birds that parental behaviour and sexuality are not necessarily incompatible. This is obviously so in those double-brooded species which begin a new clutch while the fledged young of the first brood are still being fed. The behaviour of polygynous males may also show this: for instance in the Brewer Blackbird the male may simultaneously have a female who is in the nest-building and mating phase on one site, and be helping to feed young at another nest (Williams, 1952). This is further evidence that although gonad suppression may be important in some cases, it is by no means the whole story.

It is therefore apparent that neither the presence of prolactin nor the suppression of the

gonads and of sex hormone secretion is necess-
arily essential for the occurrence of parental
responses. However, such responses cannot be
elicited at all times, or alternatively always at
equal strength, by the presentation of appro-
priate stimuli. Sometimes the response to young
may depend upon the external situation in which
they are presented. Thus the responsiveness of
hens to eggs or chicks is enhanced by keeping
them in darkness and warmth (Burrows &
Byerly, 1938; Saeki & Tanabe, 1955). The
presence of a mate or companion may be very
important: Riddle & Lahr (1944) found that
Ring Doves kept in homosexual pairs would
become broody to eggs while birds kept singly
would not. In the vole (*Microtus*), single males
will retrieve young but then may kill and eat
them, but paired males will not harm young
which they have retrieved (Frank, 1952). How
far such effects may be hormonally mediated is
not known. But even under more or less con-
stant conditions the responsiveness may vary.
Emlen (1941) found that male Tri-coloured
Redwings would not accept young before the
female had begun laying but would respond
rapidly once laying had begun, although it is not
obvious that the male undergoes physiological
changes at this time in the way that the female
must. It is often reported that incubation in birds
gradually becomes closer as hatching time nears
and the parents leave the nest less quickly on the
observer's approach, although it would seem
unlikely that the changes within the eggs are
appreciated as stimuli by the parents (e.g.
Blanchard, 1941; Farkas, 1955; van Someren,
1956). Karli (1956) tested the response of preg-
nant and lactating rats to mice which were put
into their cages, and found that just before
parturition the rats began to show maternal
responses towards the mice: this may well cor-
relate with an increase in prolactin secretion at
about the same time (Reece & Turner, 1936;
cf. also Holst & Turner, 1939). In the mouse
maternal responses possibly do not vary in this
way: Leblond (1940) found that the response to
new-born pups remained constant from just
before parturition until many weeks after,
despite the onset and decline of lactation and the
associated hormone changes, and the change in
behaviour towards a growing litter must be
ascribed to the changing stimulation which it
provides. However, an interesting set of observa-
tions by the Menzels (1953) suggests how com-
plex the interaction of internal and external
factors may be. If a bitch was given new-born

pups in place of her own older ones she re-
sponded immediately with an appropriate re-
version of behaviour, but subsequently her
behaviour changed much more rapidly, though
still gradually, than was normal or appropriate
to her foster young. Mice build especially large
'brood nests' during pregnancy and when they
have young, and the onset of this behaviour at
the end of the first week of pregnancy presents a
similar problem: this case has been very care-
fully studied by Eversmann & Koller (Koller,
1956) who found that the building of brood nests
could be induced either by treatment with
progesterone, or by the presentation of young
pups. Thus it seems that the onset of this
behaviour in early pregnancy is caused by the
rise in progesterone output at that time, while
the behaviour is maintained after parturition
(when the progesterone level falls greatly) by the
presence of the young.

This study of nest building in mice is an ex-
cellent example of how experiments on response
induction by hormone treatment can elucidate
normal changes in behaviour. In birds far too
little information is as yet available. There have
been no experiments in which the response to
standard stimuli at different stages of the
parental cycle has been thoroughly investigated,
while the experimental results on the hormonal
induction of parental behaviour are by no means
easy to interpret. The difficulty in interpreting
the experimental results comes from the variety
of treatments which may be effective. Some at
least of the effective agents are obviously not
responsible for the changes which occur norm-
ally, phenol for example. Others may act in-
directly by the stimulation of the normally
relevant hormone. This was the interpretation
given by Riddle & Lahr (1944), only unfortun-
ately they believed that prolactin was the direct
agent, and later evidence does not confirm this.
The hormones which, in one species or another,
have been found to facilitate parental behaviour
are: prolactin, progesterone, desoxycorticoster-
one, LH, androgen and thyroxine. The effect of
thyroxine tends to be considerably less than
that of the other agents, and is probably ir-
relevant to the normal situation. Desoxycorti-
costerone and progesterone are chemically very
similar and have various similar effects other
than the one in question: of these two, pro-
gesterone would seem to be the one really
relevant to the discussion. LH and androgen do
not stimulate broodiness in chickens (Nalban-
dov, Hochhauser & Dugas, 1945), androgen is

less effective than progesterone in inducing in-cubation in doves (Riddle & Lahr, 1944) and in the rat the effect of LH and androgen on maternal behaviour appears to be less than that of prolactin though possibly not significantly less than that of progesterone (Riddle, Lahr & Bates, 1942), but it must be remembered that in small mammals including rats androgen in-jection stimulates prolactin secretion (Reece & Mixner, 1939; Meites & Turner, 1947). Such considerations suggest that discussion and ex-periment should be centred mainly on the effect of prolactin and progesterone, especially as their secretion is often associated in time with parent-hood.

The influence of prolactin has already been discussed at length. It has important effects in the development of special structures associated with parental care, *viz.* the brood patches, the crops of pigeons and mammalian mammary glands. Also, at least in birds, it has a marked anti-gonad effect which may sometimes facilitate parental responses. In mammals certainly it stimulates progesterone secretion. While all these effects are probably of importance to the effect of prolactin on parental behaviour, there are suggestions that the behaviour may also be affected by prolactin in some other way in the results of experiments on castrates. Parental re-sponses which do not appear to depend upon special structures can be stimulated by prolactin in castrates, and, unless the prolactin stimulates progesterone secretion by the adrenals, this could not be mediated by the luteotrophic effect of prolactin. Experiments on castrated chickens using prolactin would be especially desirable because apparently progesterone does not stimulate parental behaviour in them (Riddle, Bates & Lahr, 1935; Nalbandov, Hochhauser & Dugas, 1945), so that the luteotrophic effect, if it exists, is irrelevant.

The influence of progesterone upon parental behaviour, although often as great as or even greater than the influence of prolactin, has been discussed less until recently. The recent studies of Lehrman (1958 a & b) suggest that it induces incubation normally in doves, while those of Eversmann & Koller (Koller, 1953; 1956) show that the pre-parturient building of brood nests in mice is probably controlled by progesterone, in both cases prolactin being without significant effect.

Even from the small amount of evidence that is available it is apparent that the control of parental behaviour may be very different from one species to another, even within a single class of vertebrates such as the birds. Comprehensive and coherent studies, such as that of Lehrman on the Ring Dove, are now needed on a variety of species.

Summary

1. The hormones most directly implicated in reproduction are the gonadotrophins and pro-lactin which are secreted by the anterior pituit-ary and the sex hormones and progesterone which are secreted by the gonads. In the in-troduction problems of interpretation, applying both to hormone experiments in general and also to the particular subject of the review, are raised. Especially in view of these, this review is in-tended more as a stimulus to future work than as an account of established conclusions—which do not, in fact, yet exist.

2. When the breeding season is just over, birds are in a state of reproductive quiescence with the gonads regressed and the pituitary refractory to stimulation. The onset of the next breeding season begins with increased pituitary activity which is stimulated by change in ex-ternal conditions, including photoperiod changes. Secretion of the gonadotrophins by the pituitary promotes growth of the gonads and the output of sex hormones by them. This in turn, together with appropriate external con-ditions, stimulates the first phase of repro-ductive behaviour including song, fighting, the setting up of territory, and courtship.

3. It is often only after a distinct courtship period that mating and nest-building behaviour are shown. As these behaviours often appear at the same time it may be supposed that both depend upon the same internal condition, and that this is probably a fairly high level of sex hormone secretion. The time of building also often corresponds with the final phase of ovarian and oviduct development prior to laying, and also with the beginning of brood patch devel-opment.

4. The physiology of laying in birds presents various interesting problems, including that of the control of serial ovulation and the control of the end of laying (*viz.* of clutch size). The end of laying in indeterminate layers involves a response to the external situation and it is suggested that prolactin may be an intermediary in this response. There is evidence of a rise in prolactin secretion during the laying period, and also it is well established that prolactin has an adverse effect upon the gonads of birds.

5. Incubation begins either during or just after the laying of the clutch. In most species the female incubates, and in many the male incubates also. A question of considerable interest is how far incubation behaviour depends upon the development of the brood patches, as well as on the appropriate stimulus situation of nest and eggs. In non-passerines there appears to be a good correlation between the development of patches and participation in incubation. However, this correlation does not hold within the *Passeres* where in various species males incubate but do not develop patches. In the female, brood patch development begins before the eggs are laid and may be ascribed to the hormonal changes necessarily taking place for the production of eggs. There is unfortunately no evidence on when exactly the brood patches of male birds begin to develop, and whether this development involves a response to the external situation. Incubation of eggs is more difficult to induce artificially than is care of young and it has been suggested that incubation results only if brood patches are developed, but there is a little evidence which conflicts with this hypothesis.

6. Prolactin has been thought to control incubation and parental behaviour because it can sometimes facilitate such responses and because there is evidence of a high rate of prolactin secretion during incubation. In fact the only evidence of prolactin inducing incubation behaviour is in the hen. The claim that it controlled incubation in the Ring Dove has not been substantiated, and it appears that in the Ring Dove progesterone controls the onset of incubation. However, progesterone is not effective in hens.

7. The pituitaries of four non-passerine species have been shown to have a high prolactin content during incubation. There is no such direct evidence in passerines and the available indirect evidence is conflicting. If incubation were associated with high prolactin secretion, the gonads would be expected to regress during incubation and this is the case in various non-passerines. In passerines, however, there appears to be no correlation between gonad condition and participation in incubation.

8. The evidence suggests that where there is a high prolactin level during incubation, the behaviour stimulates prolactin secretion rather than that the hormone controls the behaviour. If this is the case, some other function of prolactin during incubation must be postulated, and it is probable that its metabolic effects are of

importance in this context, and also the anti-gonad effect may be relevant.

9. Care of young can often be easily induced without hormone treatment both in birds and mammals. The variety of circumstances in which it can be obtained strongly suggests that there is no precise hormonal condition which is a necessary prerequisite. Except in mammals and the pigeons, where prolactin is necessary for milk production, there is no evidence of a real association in time between parental behaviour and prolactin.

However, treatment with prolactin and other agents can facilitate the induction of parental behaviour. It has been suggested that this is the result of suppression of the gonads and thus of sexual and aggressive behaviour, but the available evidence on castrated animals indicates that this is not the entire explanation. While there is some evidence that the sex hormones are antagonistic to parental behaviour, there is ample indication in wild birds that sexual and parental behaviour are not necessarily incompatible.

It thus appears that neither a high level of prolactin nor suppression of the gonads needs to occur before parental behaviour can be obtained, although both may have a facilitatory effect.

10. Various considerations suggest that future work should centre on the influence of prolactin and progesterone upon incubation and parental behaviour. Especially as there appear to be great inter-specific differences, comprehensive studies on selected species are needed.

Acknowledgments

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The above review is, to the best of my knowledge, complete till autumn 1959. I wish to draw attention here to two relevant papers which have appeared since then.

Warren & Hinde (1959) found that small doses of oestrogen had little effect on nest building in the canary but large doses could increase building behaviour markedly; progesterone had no influence upon building behaviour, whether given alone or in combination with oestrogen.

Legait (1959) has presented an extensive and interestingly conceived account of changes in hypothalamic neuro-secretion, the posterior pituitary and other endocrine organs during the annual cycle of the hen, particularly during incubation. Only part of this material has been previously published, mostly in several papers in *C.R. Assoc. Anat.* Sadly, however, this account does not altogether tally with that in the papers mentioned in the review but no mention is made of this discrepancy, and there are several inadequacies of presentation which detract from the value of the study. From the present point of view it is a great pity that nowhere are the behavioural situations defined: it is not stated how incubation was initiated or terminated, etc. None the less, the paper contains much important material on the physiology of the reproductive cycle, in particular an extensive account of anterior pituitary cytology. According to this account (disagreeing with Legait, 1955) there are

Legait, H. (1959). Contribution à l'étude morphologique et experimentale du système hypothalamo-neurohypophysaire de la poule Rhode-Island. Published thesis: Faculté de Médecine, Université Catholique de Louvain.

two peaks of neurosecretory activity in the paraventricular nucleus of the hypothalamus during incubation, at the beginning and near the end (peaks on about days 6 and 18). In both the thyroid and the adrenal there are correspondingly two peaks of activity, as there also are in the β (thyrotrophic) cells of the anterior pituitary. The conclusion drawn both from the normal relationships and from experimental results is that hypothalamic neurosecretory activity follows the activity of the thyroid and in turn influence the adrenal, this activity being most evidently related (via the posterior pituitary antidiuretic hormone) to water metabolism. During incubation the ovary regresses but then begins to grow again before incubation is terminated; there are corresponding changes in the δ (gonadotrophic) cells of the anterior pituitary which diminish in number during the first half of incubation but increase again during the latter half. It is of interest that oestrogen injections suppress the gonadotrophic cells of the pituitary. The α (acidophil) cells of the pituitary, which are associated with prolactin and growth hormone secretion, increase in number during laying and further during incubation. This increase is achieved in two bursts, during the first days of incubation and at about the 15th day. A conclusion of importance for the present review may be drawn from this study: that incubation in the hen represents neither a simple nor a constant physiological state.

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ANNUAL STIMULUS FOR SPRING MIGRATION IN BIRDS

By S. CHARLES KENDEIGH, GEORGE C. WEST AND GEORGE W. COX

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Available evidence indicates that migrant birds store fat on their bodies a few days before they migrate in the spring (Wolfson, 1954; Weise, 1956a; Odum, 1958), and become restless at night (Eyster, 1954), but permanent residents do not. The deposition of fat gives an energy reserve for northward flight that takes place normally at night. Restlessness at night in caged birds (*Zugunruhe*) is significant because it is one of the indicators that birds are in migratory condition (Weise, 1956a). A physiological difference in energy balance between migrant and resident species in the spring is thus indicated, and it is the purpose of the present study to investigate this difference and the conditions that bring the release of migratory behaviour.

The species used in the experiments were the permanent resident house sparrow, *Passer domesticus*, the yearly energy cycle of which has been studied by Kendeigh (1949) and Davis (1955) and the migrant tree sparrow, *Spizella arborea*, similarly studied by Weise (1956a) and West (1958). Although both are popularly called "sparrows" they are members of different taxonomic families: Ploceidae and Fringillidae respectively.

It is of interest that both Wallgren (1954) and Rautenberg (1957) have made comparisons of energy metabolism in resident and migrant birds, Wallgren using two species of fringillids and Rautenberg using the house sparrow and another fringillid. However, their objectives and procedures were somewhat different and in neither case did they measure nightly unrest.

Experiments Out-of-doors

Procedure

Energy intake was measured by procedures described elsewhere (Kendeigh, 1949), in which birds were placed in small individual cages and allowed to feed *ad libitum*. The caloric energy of the faeces collected at the end of three-day periods, subtracted from the gross energy intake as food, gave the energy actually metabolized during these periods. Three male and three female house sparrows were compared with three male and two female tree sparrows (one additional bird was removed when its leg was broken) with their cages placed side-by-side out-of-doors and exposed to natural changes of temperature, humidity, and photoperiod, but protected from precipitation. Each cage was fitted with a movable perch and a false bottom suspended from an electrical switch so that restless movements of the bird could be recorded continuously, using electromagnets. The three-day measurements were arranged so that two tree sparrows and two house sparrows (one tree sparrow and two house sparrows in one case) completed a run each day. The experiment was started in late February with birds freshly caught and terminated on 10th April. There was a continuous light loss of feathers in the tree sparrows, but only an occasional feather in the house sparrow.

At the end of each three-day period the birds were weighed and the amount of fat visible externally was evaluated in terms of five classes (class 1—no fat, class 5—very heavy fat). The development of nightly unrest was evaluated quantitatively by calculating the average percentage per hour of three-minute periods during which activity occurred, beginning with the first complete hour after astronomical twilight in the evening and terminating with the last complete hour before astronomical twilight in the morning.

Results

In Figs. 1 and 2, each point on the curves for metabolized energy, weight, fat, and nightly unrest represents an average for the species of the three sets of birds in overlapping three-day periods plotted on the middle day. Daily temperatures were averaged and plotted to correspond. Existence energy is the amount of energy required to maintain uniform body weight at constant temperatures under conditions permitting minimum activity. It was not measured in this experiment but calculated for each temperature from regression lines obtained by combining all the data in earlier studies on the house sparrow (Kendeigh, 1949; Davis, 1955; Seibert, 1949), KCal/bird/day=24·02—0·24t, and on the tree sparrow (West, 1958), KCal/bird/day=18·57 — 0·25t. Since there is considerable variation between individual birds, the curves for existence energy (but not for meta-
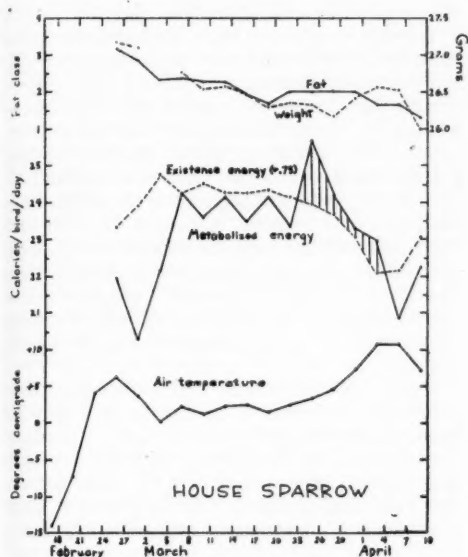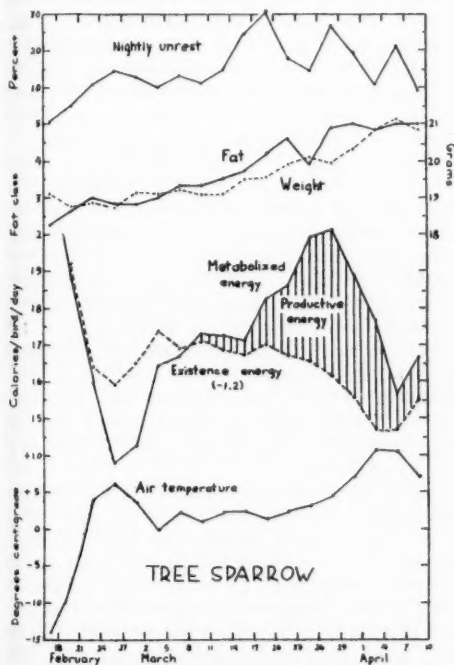
Fig. 1.

Fig. 2.

bolized energy) were adjusted to fit the particular birds used in this experiment by making them coincide with metabolized energy in the house sparrow for the three day period ending 8th March ($+0.75$) and for the tree sparrow with the mean of periods ending 5th, 8th, 11th and 14th March (-1.2) when the birds in the experiment appeared also to be metabolizing food close to an existence level.

Following the severe cold weather in mid-February, temperatures remained quite uniform until the last week of March, when a warm wave developed that extended through the first week of April. The daily photoperiod increased from less than 11 to 13 hours. During this period all but one house sparrow lost weight (Fig. 1), all declined in fat classification, and none showed any nightly unrest. In contrast, all the tree sparrows gained weight and deposited fat (Fig. 2). Likewise they all developed unrest at night. These changes are statistically significant. Both species showed an abrupt decline in daily amount of food metabolized as warm weather returned after the very cold wave in mid-February and again during the warm wave in April.

Both species started in a fat class between 2 and 3, but the house sparrow had declined nearly to Class 1 on 10th April while the tree sparrow reached Class 5 by 1st April which it then maintained. Weight tended to vary with the fat class, but not exactly. It is apparent that the house sparrow lost weight due to its metabolized energy intake from 26th February to 22nd March being less than its needs. The rise in fat class and weight from 22nd March to 3rd April coincides with a rapid decline of existence energy below the metabolized energy with the onset of high temperatures. This advantage was lost when temperatures began again to fall.

In contrast, the tree sparrow slowly put on fat and weight during the period of uniform temperature of mid-March and then rapidly as the warm wave began at the end of the month (coefficient of variation between individuals=7-8 per cent.). This corresponds with a remarkable increase of food energy metabolized in all birds (C. V.=10-14 per cent.). The food energy metabolized was much more than needed for existence. The accumulation of surplus or productive energy came at the same time as the increase in weight. This marked rise in metabolized energy is a fundamental difference between the tree sparrow and house sparrow in their energy responses to the same temperatures.

Nightly unrest in the tree sparrow was very

low during the cold weather of February, but developed appreciably with the onset of higher temperatures at the end of the month. Unrest remained at a fairly uniform level until the middle of March, after which it rose to a maximum preceding the marked increase in productive energy and deposition of fat. Unrest continued to the end of the experiment but at widely fluctuating rates.

Discussion

Fat deposition in the spring has been attributed by various authors, including ourselves, to the influence of longer days for feeding, hence greater energy intake; higher air temperatures, with consequently lower energy needs for existence; lowered standard metabolism; or direct endocrine regulation involving the gonads, thyroid, or anterior pituitary. Likewise, nightly unrest has been attributed to rising air temperatures, the excess energy (fat) that becomes available, or hormone action. These factors were thought to be interrelated in that longer days and higher temperatures in the spring brought increased productive energy, and this surplus energy that accumulated was then utilized for nightly unrest under regulation of the endocrine mechanisms (for an excellent review and citations to literature, see Farner, 1955). In our opinion, this sequence of events does not hold and we propose quite a different interrelation of factors.

Changes in weight are due primarily to deposition or utilization of fat reserves. Outside the migration period and in permanent residents generally, weight varies inversely with temperature. As a result both migrant and resident birds increase in weight in the autumn, reach a maximum in the winter, and decrease in the spring to a summer low, except that in the spring migrant species increase in weight for a short period before migration northward. This inverse relation of weight to temperature is sometimes evident even in day to day fluctuations (Baldwin & Kendeigh, 1938).

It is well established that in cold weather the rate of energy metabolism necessary to maintain body temperatures is raised, and consequently the loss of weight at night, when feeding is impossible, will be greater. This apparently stimulates increased feeding during the daytime so that there is overcompensation, that is, the increase in feeding is greater than needed just to replace the energy lost the preceding night. There is consequently a storage of the surplus intake with increase in weight.

In warm weather on the other hand, the rate of metabolism decreases, there is less loss of weight overnight and feeding is depressed. Fig. 1 shows clearly that during the warm waves in February and April, energy metabolized was less than required for existence in house sparrows so that weight decreased. There were also marked decreases in energy metabolized at these two times with the tree sparrow, which in February fell below the amount required for existence, but did not do so in April because of complicating factors shortly to be explained. Fat deposition in migrant species cannot be explained, therefore, as a result of rising temperatures during the spring.

Davis (1955) found no statistically significant difference in daily energy metabolized between house sparrows kept on a 10- and a 15-hour photoperiod. In the autumn, when both resident and migrant birds put on fat, the photoperiods are becoming progressively shorter. On short photoperiods birds simply have proportionately faster hourly rates of food consumption. Therefore, contrary to many statements in the literature, birds do not necessarily eat more per day in the spring and fat deposition cannot be due simply to increase in length of photoperiods. It is significant, as will presently develop, that the increased feeding in experimental birds placed in longer photoperiods (King & Farner, 1956) is joined with development of nightly unrest.

The fact that in the autumn, nightly unrest develops only in migrant species, although both migrants and residents put on fat, indicates that nightly unrest is not due simply to surplus energy being available. Likewise, no one has demonstrated that endocrines by themselves are capable of initiating nightly unrest in a normal manner.

What then is the course of events that induces nightly unrest and fat deposition in the spring preparatory to migration? As a working hypothesis we propose that fat deposition is a result of and not the cause of nightly unrest and that nightly unrest is directly induced by changes in the environment.

Restlessness at night, once induced, necessitates wakefulness, muscular activity, and higher body temperature, compared with birds at repose. The increased metabolism that consequently occurs uses up energy and accelerates weight loss at night in the same manner as does low temperature. Increased energy intake follows with more rapid feeding during the daytime. This was shown for migrant European species over

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twenty years ago (Merkel, 1937). What was not demonstrated, however, was that, as under the influence of cold, so also under the influence of nightly activity, there is overcompensation in feeding, and the surplus of energy that accumulates is stored as fat. The occurrence of nightly unrest in the tree sparrow and not in the house sparrow is the only factor brought out in this experiment that can explain the increase in metabolized energy in the former and not in the latter in the face of at first constant and later rising temperatures, contrary to the way that it varies with temperature when nightly unrest is not involved (West, 1958). With nightly unrest thus shown to be of such importance it is obvious that all experimental studies on the effect of environmental, endocrine, or other conditions on fat deposition or energy metabolism in migrant birds are open to question unless this factor is measured and properly evaluated.

Since birds can gain weight only when energy intake is greater than energy utilization and can lose weight only when utilization is greater than the intake, it may be that the *principle of over-compensation in rate of feeding* may be of wide application during periods of energy stress. This appears to be the explanation for the inverse correlations between weight and air temperature and we believe we have here demonstrated it also for fat deposition as the result of nightly unrest.

Initiation of Nightly Unrest

Eyster (1954) reviewed the early literature and found the evidence inconclusive for a clear correlation between temperature and nightly unrest. Likewise in his own experiments with three migratory fringillid species, he found considerable fluctuation in nightly unrest independent of temperature. Weise (1956a) found that in three migratory fringillids confined in small cages out-of-doors that nightly unrest during the spring increased with the passage of warm fronts and high environmental temperatures and was inhibited by cold fronts and low temperatures. He also demonstrated experimentally (Weise, 1956b) that nightly unrest does not develop in castrated birds but will gradually appear under long photoperiods if this castration is incomplete and testicular tissue regenerates. This supports Rowan's theory (1932), propounded several years ago, of a causal relation between lengthening photoperiods, developing gonads, and spring migration (but see Millar, 1959).

In experiments by Farner & Mewaldt (1955)

with the migratory white-crowned sparrow, *Zonotrichia leucophrys*, birds confined to mean temperatures of —4 to +1°C. and with natural photoperiods increasing from approximately 10·5 to 12·5 hours did not develop nightly unrest although another group of birds subjected to the same photoperiods but held at a temperature of 21°C. did do so. In both groups the weight of the testes increased between 2x and 3x during the last two weeks of the experiment. In two other groups of birds subjected to 15 hour photoperiods, both developed nightly unrest but more extensively so in the group held at 22°C. than in the one held at —2° to +3°C. There was extensive enlargement of the testes in both groups.

Experiments

In order to evaluate the relative role of temperature and photoperiod in initiating nightly unrest a series of three experiments under controlled conditions was undertaken during the spring of 1959 with tree sparrows, freshly caught.

In the first experiment, 10 birds (5 males, 5 females) were held at a temperature of —4°C. and the photoperiod raised gradually from 10 hours on 6th January to 15 hours on 21st January. It was then held constant until 16th February, after which it was again raised gradually to 19 hours by 24th February. Nightly unrest was negligible until the photoperiod reached 17 hours when it began to develop, attaining a peak of about 34 per cent. on the third night after the photoperiod reached 19 hours. Apparently increasing photoperiods by themselves are sufficient to initiate nightly unrest. Low temperatures do not prevent nightly unrest from occurring, but at low temperatures much longer photoperiods are required. In the previous experiment out-of-doors (Fig. 2), tree sparrows had developed nightly unrest (10-15 per cent.) with natural photoperiods around 11 hours at this same time of the year, late February, but at the higher temperatures of 0° to +6°C.

In the second experiment, 4 male and 6 female birds were held on a constant photoperiod of 10 hours and the temperature was raised. The temperature was initially constant at +3°C. from 29th January to 15th February as the freshly caught birds declined in weight from 20·8 to 18·2 g. and in fat class from 4·6 to 2·7. The temperature was then increased gradually and progressively until it reached 20°C. on 7th March. The birds' weight remained the same, fat class recovered slightly (to 3·8), and nightly unrest fluctuated sporadically throughout the

period between zero and only 2 per cent. Control birds out-of-doors show 10-15 per cent. nightly unrest at this time of year at much lower temperature, but on a photoperiod of about 11·3 hours (Fig. 2). Although the gonads of the experimental birds were not examined, in all probability they remained in the resting winter condition. Apparently a rise in temperature on a photoperiod too short to be stimulating to gonad development cannot initiate sustained nightly unrest, and without nightly unrest there is no significant increase in weight or fat.

The temperature to which the birds were exposed was then lowered to +2·5°C. by 12th March and held at this level until 1st April to allow the birds to become readjusted to simulated winter conditiods. In this third experiment the photoperiod was held at 12·5 hours. Temperature was raised first to 5·5°C., 2nd to 6th April, then to 10°, 8th to 13th April, then to 15°, 14th to 26th April, and finally to 20°, 27th April. Nightly unrest fluctuated between zero and 1·5 per cent. at 2·5°C., even though at this time of year birds out-of-doors reach a peak of activity (Fig. 2). Nightly unrest began to show signs of increasing when the temperature was raised to 5·5°C. This increase became definite at 10°C., 6·5 per cent., and reached a peak of 20 per cent. eight days after the temperature was raised to 20°C. It is possible that nightly unrest would have developed more rapidly had not a light moult been induced. The birds increased steadily in average weight from a low of 18·1 g. on 20th March to a high of 21·2 g. on 4th May. Fat class increased during the same interval from 4·2 to 5·0. However, the presence of the feather moult confuses the relation between nightly unrest and fat deposition. Autopsies made on 28th May showed the testes enlarged to at least one-half maximum size. It thus appears that the onset of nightly unrest was initiated by simultaneous moderate increases in both photoperiod and temperature in a manner similar to the way that it is under natural conditions. The results of these three experiments are in harmony with those of Farner & Mewaldt (1955) above mentioned.

The Stimulus

For birds wintering north of the Tropics, the factors that are responsible for initiating spring migration northward are complex and interacting. We present the following summary of what we consider the sequence of events leading to the migratory stimulus, subject to later revision or more complete verification. (1) Increasing photoperiods are important, not because they give longer daily periods for activity and feeding but perhaps because they bring (2) the recrudescence of gonadal activity. (3) Increasing temperatures not only diminish the energy requirements for existence but perhaps more significantly they along with increasing photoperiods induce (4) nightly unrest on the part of the birds. This nightly unrest results in increased rate of feeding beyond the needs of existence so that (5) fat deposition occurs. This reserve of fat is essential to provide energy for the northward flight that occurs at night. All this puts the bird into proper physiological and psychological readiness for migration. Nightly unrest continues to augment with increasing favourable conditions until its intensity reaches a requisite threshold. Probably then the actual stimulus that releases the migratory behaviour is (6) the passage of warm fronts with clear weather and favourable winds.

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IMMOBILITY REACTIONS (FEAR) OF DOMESTIC FOWL AS A FUNCTION OF AGE AND PRIOR EXPERIENCE

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Immobility reactions to human handling, also frequently called animal hypnosis or trance, have been the subject of investigations and essays since 1646. Armstrong (1942), Weitzenhoffer (1953) and Gilman & Marcuse (1949) present summaries of some of the literature on the subject. The immobility reaction has several interesting and rather consistent properties: (*a*) it is associated with handling and particularly holding or stroking of the animal (Gilman & Marcuse, 1949; Ratner, 1958); (*b*) it has durations from less than a minute to beyond an hour (Armstrong, 1942; Bayard, 1957); (*c*) analgesia occurs during immobility (Gilman & Marcuse, 1942; Ratner, 1958); (*d*) it is found to occur among a large number of species of vertebrates and invertebrates (Armstrong, 1942; Gilman & Marcuse, 1949).

Since the immobility reaction to handling was labelled as animal hypnosis, researchers tended to set it apart from other events which were being studied, although recent considerations of the reaction (Armstrong, 1942; Gilman, Marcuse & Moore, 1950; Ratner, 1958) have emphasized its relationship to fear. However, so much remains to be known about the characteristics of the reaction that a rigid theoretical commitment to the fear hypothesis does not yet seem possible. The present study was conceived loosely around the fear hypothesis, but was designed primarily to investigate systematically several variables which prior research suggested as relevant.

Portions of a study by Gilman, Marcuse & Moore (1950) indicated that daily handling and testing of adult chickens reduced the durations and incidence of immobility. Vogel (1950) reported a field study of turkey vultures in which the adult bird in the nest became immobile when touched and prodded with a stick, while the young did not show this reaction. Thus, the present investigation was designed to determine the effects of age and prior testing on the durations and incidence of immobility reactions with domestic chickens.

Experiment 1. Immobility from 10 to 66 Days of Age

Subjects

The subjects for this study were 220 White Leghorn cockerel chicks obtained from a local hatchery approximately 30 hours after having been removed from the incubator. The results are based on 203 birds, since a total of 17 died, escaped, or became ill during the course of this study. The birds were maintained on Michigan State University chick starter, a complete food, during the course of the study. This food and water were available at all times.

Apparatus

The birds were shipped in standard cardboard shipping boxes and were transferred immediately into brooders, each tier of which was 3 ft. $\times$ 3 ft. $\times$ 1 ft. A heated fabric top extended across one-third of the tier while the remaining area had a wire top. Food and water trays extended around the non-heated perimeter. The birds were housed in these units for the duration of the study, although the heated elements were turned off after two weeks. The brooders were located in a large experimental room which contained many other similar brooders with chickens and turkeys of various ages.

Testing was carried out on a large table located 10 ft. from the brooders which housed the birds. The top of the table was covered by cardboard and a cardboard box was placed on this so that the working area was divided into two sections, allowing two birds to be tested at one time.

An exhaust fan was running continuously during testing. Stop watches were used for timing durations of immobility and intertrial intervals.

Procedure

Several days after the birds arrived, each was removed from his brooder, banded on the wing with a numbered metal tag, and assigned haphazardly to one of two groups. Forty-five birds were assigned to the repeated test group, Group RT, and were put together into one brooder. The

remaining 165 were assigned to the single test group, Group ST, and were divided equally between two other brooders. Food, water, light, ventilation, etc., were similar for the three brooders, although during the first few weeks, more birds were housed in the brooders for Group ST than in the brooder for Group RT. The numbers of birds in each brooder were well within the limits suggested for such living space.

The birds in the repeated test group, Group RT, were tested when ten days old and then retested at weekly intervals for eight consecutive weeks. Those in the single test group, Group ST, were divided into subgroups with 20 birds in a subgroup. A different subgroup was tested at each weekly session and then discarded. Thus, at each test session the 45 birds in Group RT and a subgroup of 20 from Group ST were run. At the first session, when the birds were ten days old, only Group RT was run since they had had no prior test experience and could provide evidence on the effects of age unconfounded by prior test experience. Tests were conducted from approximately 6.30 a.m. until noon during July, August and early September.

Since this study was designed in part to extend Gilman, Marcuse & Moore's investigation (1950) regarding the effects of prior testing, their handling procedure for testing birds was followed. Specifically, the weekly tests for Group RT were conducted in the following way. Two birds were removed from the brooder and each was placed in the cardboard box on the testing table. After 15 seconds, the birds were removed from the box and placed, one on each side of it, so they could not see each other. They were lightly held in a standing position for 5 seconds, after which the bird in the right hand was turned over on its left side by a rapid movement of the experimenter's right hand and the bird on the left side was turned over on its right side by a rapid movement of the experimenter's left hand. Both birds were held in their lateral positions for 15 seconds with decreasing pressure during this time. At the end of 15 seconds the experimenter slowly removed his hand. In keeping with criteria used by Gilman, Marcuse & Moore (1950) immobility was scored if the bird remained in the lateral position for more than 5 seconds. Duration of immobility was measured from the time the experimenter's hand was lifted *until the bird got to its feet*. Small movements of the bird's head and feet frequently occurred but were not counted as the termination of the reaction. An arbitrary maximum duration of 4 minutes of immobility was established. If it had not risen to its feet after 4 minutes, it was lifted, righted and allowed to stand on the table for several seconds. In either case, however, the bird was returned to the cardboard box for 15 seconds, and then retested as above. This was repeated one more time for a total of three trials per test session for each bird.

The birds in Group ST were treated in an identical manner, except that they were destroyed after the third trial instead of being placed in a holding brooder. This accounted for the gradual reduction in the size of the groups in each brooder containing Group ST.

The side on which the birds were placed for testing was the same on a given test day, but varied haphazardly from one day to another for Group RT. One experimenter tested all the birds while the other recorded incidence and durations of immobility.

Results

The medians of the total durations of immobility at each age for Groups RT and ST are shown in Fig. 1. Specifically, for each group the numbers of seconds of immobility for the three trials of each session were summed for each bird, and the medians were then obtained for the session. It can be seen from Fig. 1 that birds in Group RT responded almost maximally (maximum was 720 seconds per session) at ten days of age but durations fell markedly so that by 66 days of age, the median total duration for Group RT was only 14 seconds.

Statistical analyses of the changes in total durations of immobility for Group RT alone were made by means of chi square tests which showed that 29 of the 44 birds had shorter durations on the second session than on the first. This yielded a significant value of chi square (chi-square $= 5 \cdot 25$, df $= 1$, $p = < \cdot 05$). The comparison between the second and ninth sessions (ages 17 and 66 days) indicated that 41 of the 43 birds had shorter durations of immobility on the ninth than on the second session, which yielded a highly significant value of chi square (chi square $= 35 \cdot 37$, df $= 1$, $p < \cdot 001$).

In terms of susceptibility to immobility, birds in Group RT responded on $97 \cdot 7$ per cent. of the trials in the first two sessions. During the first two sessions, all birds showed some response while by the last session only 31 of the 45 birds responded.

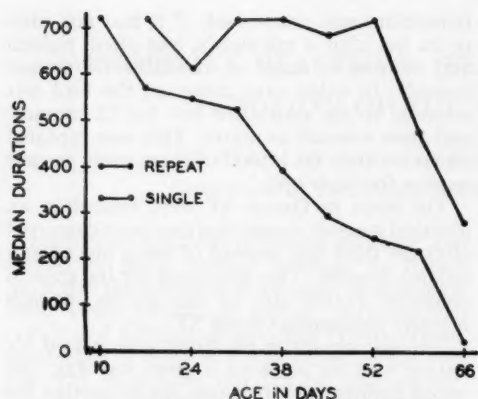The median durations of immobility for Group ST, the single test group, were quite

Fig. 1. Relationship between age and median durations of immobility for birds tested repeatedly, Group RT, and birds tested only once, Group ST, in Experiment I.

different from those of Group RT, as seen in Fig. 1. Median durations remained very close to maximum until the birds were 59 days of age, after which the median dropped to 315 seconds at 66 days of age. The drop in the curve at 24 days of age was tested by a Mann-Whitney U test (Siegel, 1956, p. 116) and was found to be a non-significant change as compared with responses at 17 days of age. However, tests of durations of immobility, comparing responses at 17 days of age with those at 66 days, indicated a very significant decrease in durations ($U=60$, $N_1 = N_2 = 20$, $p < \cdot001$). This indicates, of course, that the animals were not responding as strongly as they had earlier in life. Susceptibility for Group ST as indicated by the percentage of trials on which responses occurred was nearly maximal at 17 days of age (responses on 98·7 per cent. of the trials). By 66 days of age, however, responses occurred on 73·3 per cent. of the trials with two of the 20 birds not responding.

Statistical comparisons between groups of RT and ST yield the results suggested by examination of Fig. 1. That is, the groups differed significantly at ages 17 days as tested by Mann Whitney U test for large samples ($Z = 2\cdot85$, $N_1 = 20$, $N_2 = 44$, $p < \cdot01$) and of course differed significantly from each other at 66 days of age.

The following behaviours were frequently observed during immobility: raising and lowering of heads, blinking, vocalizing the distress cry when young, defaecating, and flexing or extending of legs. These responses were made rather slowly and were not associated with the ter-

mination of immobility. Several birds selected haphazardly from Group ST were left in their immobile state after the last trial of a session and were found to remain immobile for an average of 75 minutes.

Prior research with rabbits in a quiet room (Ratner, 1958) showed that immobility was always terminated by loud sounds. However, in the present study loud and sudden sounds seemed only fortuitously to be associated with the termination of immobility. Close observation for behaviours or external conditions associated with the termination of immobility yielded no clues. Birds just suddenly jumped up and moved away.

Experiment 2. Immobility from Two to Twelve Days of Age

Since the results of Experiment 1 did not indicate a time early in the birds' lives when they did not show immobility to handling, this study was designed to test for it from two to twelve days of age, using the same experimental design as was used in Experiment 1.

Method

Subjects

The subjects for this study were 83 pure-bred, male Cobb chicks obtained from a local hatchery when approximately 30 hours old. Cobbs were used due to the unavailability of Leghorns, but there was no reason to think they should differ much. Chicks were maintained on Michigan State University chick starter, which was available with water at all times.

Apparatus

The brooder for housing the birds was identical with the one used in the first study. The birds were housed and tested in a hut in which no other birds were kept at the time this study was conducted. Testing was conducted in a 12 ft. × 12 ft. cubicle, next to the cubicle containing the brooder. As in Experiment 1, tests were conducted on a large table covered by cardboard and carboard boxes were used to hold the birds between trials. Stop watches were used to time durations of immobility and intertrial intervals.

Procedure

The procedure used in this study was the same as that used in Experiment 1 with several minor exceptions. The birds in this study were tested for the first time within several hours after

having been received from the hatchery and they were tested one at a time, rather than in pairs.

Briefly, the procedure involved dividing the 83 birds at random into the repeated test group, Group RT, and the single test group, Group ST. Group RT contained 23 birds which were tested when two, three, four, five, seven, nine, and twelve days of age. Group ST contained a total of 60 birds divided into subgroups of ten. A different subgroup was tested at each age at which Group RT was tested and then the subgroup was discarded. An ST subgroup was not run at two days of age, since the control for prior testing was not necessary for the first test for Group RT. Each bird in both groups received three trials at the test session for a given age with the same procedure and criteria as had been used in Experiment 1.

Results

The medians of the total durations of immobility at each age and the percentages of birds responding on one or more trials at each age are shown in Fig. 2. The total duration of immobility at a given age for a single bird was obtained by summing the immobility durations of greater than five seconds for each of the three trials at the given age. It will be remembered that an arbitrary maximum duration of 240 seconds was set for each trial, thus making a maximum of 720 seconds for a given session.

Examination of the median durations of immobility shown in Fig. 2, indicates that the median duration for Group RT rose above zero
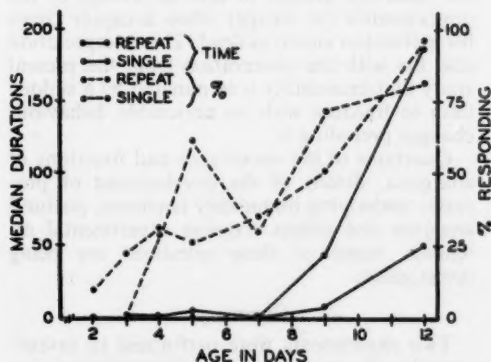
by nine days of age and rose even higher by twelve days of age. Since 22 of the 23 birds in Group RT showed longer durations on day twelve than on day three, it was concluded from the binomial test that the change was highly significant. A similar comparison between subgroups from Group ST for days three and twelve by the Mann-Whitney U test indicated the change in immobility durations for Group ST was highly significant ($U = 10$, $N_1 = N_2 = 10$, $p < .01$).

Comparisons between Groups RT and ST by Mann-Whitney U tests for large samples at nine and twelve days of age indicated that the groups did not differ significantly in durations of immobility on day nine, however, the groups did differ significantly from each other by twelve days of age ($Z = 1.79$; $N_1 = 23$, $N_2 = 10$; $p < .05$).

Examination of the curves in Fig. 2 showing the relationship between age and percentage of birds responding on at least one trial of the session suggest: (a) the incidence of immobility reaction gradually increased as the birds aged and (b) the groups were not differentiated by this measure, although they were by the more sensitive measure of duration of immobility.

A chi-square test of independence was computed to determine the statistical significance of the relationship between age and incidence of immobility for Group ST. The frequencies of birds responding and not responding at three age levels were determined and the chi-square was then computed. The frequencies of birds responding were as follows: three and four days, three birds; five and seven days, nine birds; nine and twelve days, 15 birds. A highly significant value of chi-square was obtained (chi-square $= 14.56$, df $= 2$, $p < .001$), and the hypothesis of independence was rejected.

Observations of the behaviours of the birds prior to, during, and at the termination of immobility verified the observations made in Experiment 1. In addition it was noted, when the birds were from seven to twelve days of age, that on trials when they remained immobile it could be predicted from the fact that they became limp under the experimenter's hand and their legs slowly extended. This posture is shown in Gilman, Marcuse & Moore's photographs of adult birds in the lateral position (1950). Testing of ten birds during immobility under darkened conditions indicated the presence of pupillary contraction and dilation with alternate conditions of light and dark for every bird so tested.

Fig. 2. Relationship between age and median durations of immobility (left ordinate) and percentages of birds showing immobility (right ordinate) for birds tested repeatedly, Group RT, and birds tested only once, Group ST, in Experiment II.

As in Experiment 1, close observation for re-
sponse or stimuli associated with the spontan-
eous termination of immobility yielded no
systematic clues.

Discussion

The results of Experiments 1 and 2 considered
jointly, clearly indicated that both age and re-
peated testing affected the duration and in-
cidence of immobility reactions of chickens.

Immobility reactions were at very low strength
in terms of incidence and duration for the first
week of the birds' lives and then rapidly came to
high strength so that by the beginning of their
second week, the responses occurred with more
than 90 per cent. of the birds, considering both
experiments together. The incidence and dura-
tion of immobility remained at maximum
strength for Group ST until the birds were 59
days of age, at which time the durations de-
creased. While this may have been a function of
age changes, the decrease is consistent with the
fear hypothesis. That is, the birds in Group ST
had had a large number of casual contacts with
humans which occurred as a function of main-
tenance of the birds. It is hypothesized that these
casual contacts reduced the fear reactions as
taming was shown to do by Gilman, Marcuse &
Moore (1950).

The data showing the course of development
of the immobility reactions of the young chick-
ens, nedifugus birds, runs parallel to observa-
tions of the development of fear reactions in
other species of nediculus birds. Nice (1943, p.
53) reports that fear as shown by cowering and
immobility occurs within six to ten days of age
for such species as the Song Sparrow, Catbird,
Cowbird, Common Redstart and others. She
also summarizes some results (1943 p. 53), which
indicate that the appearance of fear reactions is
associated with the development of physical in-
dependence. The development of physical in-
dependence also occurs to some extent with the
domestic chick at seven to fourteen days of age.
It is a time of establishing body temperature
control mechanisms (Sturkie, 1954, p. 122) and a
time at which heart rate begins to decrease and
approach the adult level (Ringer, et al., 1957).
Since the data on development of immobility
reactions to handling parallels the data on de-
velopment of fear reactions, it lends some sup-
port to the hypothesis that immobility to handling,
called animal hypnosis by Gilman, Marcuse
& Moore (1950), is a profound fear reaction.

The effect of prior testing was to reduce sig-
nificantly duration of immobility at all ages.
Prior testing showed its effects very rapidly so
that by twelve days of age, after six prior sessions,
the reaction of Group RT was significantly re-
duced in Experiment 2. The reactions of Group
RT in Experiment 1 with older birds, indicated
that significant reduction required only one prior
session. The finding that a few widely spaced
experiences with testing for immobility can
greatly reduce the duration and incidence of
immobility suggests an explanation of the
apparent paradox noted by Marcuse & Moore
(1949), and Weitzenhoffer (1953), that great in-
dividual differences in immobility reactions occur
within a species and for a given animal from
time to time.

The present interpretation of the effects of
prior testing is not in terms of the testing *per se*,
but in terms of the effects of handling associated
with testing. In this study each bird was picked
up and put down 13 times during each session.
Thus, it is assumed that each was gradually
being tamed by handling associated with testing
and thus reacted by immobility to a lesser
extent. This is consistent with Gilman, Marcuse
& Moore's finding (1950) that taming in advance
of testing reduced the reaction.

The adaptive significance of immobility at
times when danger is near is well accepted and
fully documented. However, the significance of
immobility when 'handled by a predator' is
less widely noted or acknowledged. Armstrong
(1955, p. 249) indicates a function for immobility
with handling when he suggests that it enables
the immobile animal to take advantage of the
opportunities (to escape) when a captor treats
his motionless victim as dead. This interpretation
also fits with the observation from the present
study that immobility is terminated by a sudden
dash to freedom with no noticeable behaviour
changes preceding it.

Questions of the occurrence and functions of
analgesia, details of the development of pro-
cesses underlying immobility reactions, postural
analyses and others requires experimental at-
tention. Some of these questions are being
investigated.

Summary

Two experiments were performed to investi-
gate the effects of age and prior testing on im-
mobility reactions of chickens from two through
66 days of age. In each experiment, one group
was tested repeatedly while different subgroups
were tested only once at the ages of the repeated

test group and then discarded. Tests were conducted by turning each of the 305 birds on its side and holding it down for 15 seconds after which the immobility reactions were observed and timed. The immobility reaction was found to be virtually absent until seven to ten days of age, after which the response reached and maintained the arbitrary maximum duration of twelve minutes until birds were 59 days of age. Prior testing and the handling associated with it significantly reduced duration and incidence of immobility at all ages after nine days. The results are interpreted in terms of immobility as part of a fear reaction.

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EFFECT OF COMB AND WATTLE REMOVAL ON THE SOCIAL ORGANIZATION OF MIXED FLOCKS OF CHICKENS

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Although many experiments have been conducted to determine the effects of surgical removal of the comb and wattles (dubbing) on production characteristics in chickens, only meagre information is available on the influence of these appendages on social behaviour. Allee *et al.* (1939) and Collias (1943) reported that comb size was an important factor in the success of initial encounters between hens. Guhl & Ortman (1953) showed the importance of visual patterns in recognition among chickens. They found that disguised features about the area of the head and neck were more effective in producing a loss of recognition than alterations made to areas of the trunk.

Hale (1956) reported that in social interactions between breeds of chickens, behavioural responses were based on breed recognition rather than on individual recognition. Individuals placed in a flock of another breed were accepted as dominant birds without challenge if members of that breed had previously defeated or dominated members of the other breed in that flock. Tindell & Craig (1959) however, observed that recognition in flocks of mixed breeds was mainly on an individual rather than breed basis.

Cole & Hutt (1954) in a study chiefly concerned with the effects of dubbing on egg production suggested that dubbed birds were less subject to an established peck-order.

The purpose of the experiments herein reported was to determine the influence of dubbing on the social behaviour of chickens intermingled in large and small flocks with undubbed birds. A second phase of this paper is to present a statistical method which is applicable to data obtained in behavioural studies.

Experiment I

Procedure

Commercial hybrid chickens were hatched April 15th, 1958, brooded in confinement until 9 weeks of age and then placed on range. At 20 weeks of age 340 pullets were randomized into

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four equal sub-class groups designated as A, B, C and D. At this time the comb and wattles were removed from 2 groups (A and C) and left intact in the other 2 groups (B and D). Groups A and B were then housed as a single flock in a 15 ft × 29 ft. pen located in a conventional laying house while Groups C and D were housed as a second flock in a pen located in a modified solar house. Both pens were of the same size and each was located in the northeast corner of its respective house. The same feeding, watering, nesting and roosting allocations were provided in each pen.

Females in each flock were observed in the area of the combination feeder-waterer. This area was selected since it was one where activity between birds was greatest. The frequency of encounters (fights, pecks, threats and avoidances) during a 50-minute period in the late afternoon was recorded separately for dubbed and non-dubbed birds. Observations were made four times each week until 44 days after the flock was housed, then weekly until 93 days after housing. From the 93rd day after housing to the end of the experimental period, 282 days after housing, bi-weekly observations were made.

Analysis and Results

An analysis was necessary to determine if there were any significant differences within each period of observation for the numbers of encounters won by the following: dubbed over dubbed, dubbed over controls, controls over controls, and controls over dubbed. In the past, data of this nature had been analysed by the goodness of fit test. To do this it was necessary to hypothesize a set of probabilities associated with each class and many times the hypothesized set consisted of chance occurrences due to the lack of information available to the investigator. If the hypothesized set of probabilities were rejected the investigator could only conclude that one or more of the hypothesized values of the parameters were incorrect. If the hypothesized values were not rejected the experimenter was only able to conclude that this set was one of an infinity of sets of values for the parameters,

which could be specified, but which would not lead to rejection either.

Several attempts have been made to obtain more information from data of this nature. However, most of the methods developed thus far are dependent on the ordering of the classes. The method described below, developed by Hurst & Queensberry (1958) should prove very helpful to workers faced with the problem of analysing data obtained from behavioural experiments. The data obtained in Experiment I have been submitted to this technique and the results are given by use of lower case letters. Hurst & Quesenbury called their technique "Asymptotic simultaneous confidence intervals for the probabilities of a multinomial distribution."

In this paper only the notation, properties and method of computing the intervals will be presented because the theory is presented in the Hurst & Queensberry report. The following notations will be adhered to in this paper:

N is the number of observations in the sample.

n is the number of observations in the ith cell,

π_i is the probability that any observation will fall in the ith cell,

k is the number of cells, and

$P_i = n_i/N$ is the proportion of observations that fall in the ith class.

The π_i's are assumed unknown but $\sum_{i=1}^{k}\pi_i = 1$.

The $100 (1-\alpha)\%$ confidence interval is given by

$$\pi_i = \frac{\chi^2_{\alpha, k-1} + 2n_i \pm \sqrt{\chi^2_{\alpha, k-1}[\chi^2_{\alpha_{k-1}, } + 4n_i(N-n_i)/N]}}{2(\chi^2_{\alpha, k-1} + N)}$$

The desirable properties of these intervals are:

1. the intervals will always exist,

2. the intervals will converge upon P_i from both the left and the right as the sample size increases,

3. intervals whose corresponding $P's$ are equal distances from 1/2 will be of equal length.

4. the intervals will always lie entirely in the closed interval $[o, 1]$ and contain the point P_i.

To illustrate the computations involved consider the data from day 32 in the conventional laying house, as presented in Table II, and find a 95 per cent. confidence interval. Table I was designed with each stage of the calculation indicated at the top of each column. Obtain $\chi^2_{.05, 3} = 7.81$ from a table of χ^2 values and follow the computational procedures outlined in Table I.

Columns (10) and (11) give the lower and upper limit respectively for π. By observing that there is overlapping of intervals for D→C and C→C as well as for D→D and C→D one may now write:

D→D	C→D	C→C	D→C
40[a]	65[a]	10[b]	4[b]

and state that any two members which do not have the same superscript are significantly different at the 5 per cent. level.

Data presented in Table II show that dubbed chickens were significantly subordinate to non-dubbed chickens during every observation period. After flock organization became more complete this difference became even more pronounced and it appeared that there was a social stratification with the undubbed birds maintaining the higher level of the social order. This stratification was so pronounced that 107 days after housing the dubbed birds were not observed to win a single encounter over a non-dubbed bird. It may also be observed in Table II that the frequency of encounters per observa-

Table I. Computational Procedure for the Confidence Limits.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	n_i	$N-n_i$	$4n_i(N-n_i)$	$(3)/N$	$\chi^2[\chi^2+(4)]$	$\sqrt{(5)}$	χ^2+2n_i	$(7)-(6)$	$(7)+(6)$	$\frac{(8)}{2(N+\chi^2)}$	$\frac{(9)}{2(N+\chi^2)}$
D→D	40	79	12,640	106·218	890·56	29·84	87·81	57·97	117·65	·228	·464
D→C	4	115	1,840	15·462	181·75	13·48	15·81	2·33	29·29	·009	·115
C→D	65	54	14,040	117·983	982·44	31·34	137·81	106·47	169·15	·420	·667
C→C	10	109	4,360	36·639	347·15	18·63	27·81	9·18	46·44	·036	·183

D→D Dubbed over dubbed.   C→C Control over control.
D→C Dubbed over control.   C→D Control over dubbed.

Table II. Comparisons of Frequency of Encounters Between and Among Dubbed and Non-dubbed Birds by Flock and Days.

Days from housing	Conventional laying house				Modified solar house			
	D→D	C→D	C→C	D→C	D→D	C→D	C→C	D→C
32	40[a]	65[a]	10[b]	4[b]	23[a]	29[a]	9[b]	3[b]
33	53[a]	64[a]	12[b]	5[b]	27[b]	57[a]	6[c]	6[c]
36	49[a]	60[a]	5[b]	1[b]	22[a]	36[a]	4[b]	2[b]
37	49[a]	52[a]	8[b]	4[b]	22[a]	43[a]	2[b]	2[b]
38	63[a]	77[a]	9[b]	1[b]	23[ab]	41[b]	6[ac]	2[c]
39	55[a]	64[a]	9[b]	2[b]	13[b]	52[a]	9[b]	1[b]
43	50[a]	56[a]	7[b]	1[b]	26[a]	29[a]	3[b]	2[b]
44	53[a]	71[a]	7[b]	3[b]	32[a]	35[a]	2[b]	1[b]
51	40[a]	41[a]	6[b]	2[b]	29[a]	39[a]	3[b]	0[b]
58	36[a]	44[a]	4[b]	2[b]	26[a]	43[a]	4[b]	0[b]
65	38[a]	41[a]	4[b]	0[b]	28[a]	35[a]	4[b]	1[b]
72	25[a]	17[a]	6[b]	0[b]	24[a]	27[a]	2[b]	2[b]
79	24[a]	18[a]	3[b]	0[b]	18[a]	24[a]	4[ab]	0[b]
93	24[a]	19[a]	4[b]	0[b]	18[a]	16[a]	1[b]	2[b]
107	19[a]	9[a]	1[b]	0[b]	14[a]	7[a]	3[ab]	0[b]
121	17[a]	14[a]	2[b]	0[b]	16[a]	12[a]	4[ab]	0[b]
135	15[a]	15[a]	3[ab]	0[b]	16[a]	14[a]	4[ab]	0[b]
149	20[a]	12[a]	5[ab]	0[b]	19[a]	15[a]	4[b]	0[b]
163	14[ab]	26[a]	4[bc]	0[c]	14[a]	16[a]	4[ab]	0[b]
184	15[a]	14[a]	6[ab]	0[b]	18[a]	11[a]	2[b]	0[b]
198	15[a]	15[a]	5[ab]	0[b]	12[a]	14[a]	4[ab]	0[b]
212	17[a]	8[a]	4[ab]	0[b]	16[a]	14[a]	4[ab]	0[b]
226	13[a]	12[a]	5[ab]	0[b]	15[a]	15[a]	5[ab]	0[b]
240	11[a]	8[a]	3[ab]	0[b]	18[a]	6[a]	4[ab]	0[b]
254	11[a]	8[a]	2[ab]	0[b]	16[a]	10[a]	4[ab]	0[b]
268	19[a]	8[a]	5[ab]	0[b]	17[a]	12[a]	8[ab]	0[b]
282	16[a]	13[a]	5[ab]	0[b]	15[a]	17[a]	7[ab]	0[b]

Any two numbers on the horizontal which do not have same superscript are significantly different at the 5 per cent. level based on the simultaneous confidence interval technique.

D→D Dubbed over dubbed.   C→C Control over control.
D→C Dubbed over control.   C→D Control over dubbed.

tion period among dubbed females was greater throughout the entire experiment than for any period among the undubbed females. In 29 out of 54 comparisons the difference between the number of encounters among dubbed birds was significantly greater than the number of encounters among undubbed birds.

Experiment II

Procedure

To ascertain further the influence of dubbing on social behaviour, 48 White Plymouth Rock pullets, 328 days of age, were randomly divided into 8 flocks of 6 birds each. After a peck-order was determined in each flock, combs and wattles were removed from 3 pullets within each flock. In 4 of the flocks the number 1, 3 and 5 ranked birds were dubbed while in the others the number 2, 4 and 6 ranked birds were dubbed. The dubbing operation was performed at night. In 4 flocks (Flocks 5-8) the chickens were placed in a neutral pen immediately after dubbing, while in

the other 4 (Flocks 1-4), dubbed and non-dubbed females were not placed together in the neutral pens until 3 weeks post-dubbing. Peck orders in each flock were obtained weekly for 6 weeks after the pullets were placed in the neutral pens to determine the effect of dubbing on the social order of each flock.

Results

Peck-orders presented in Table III show a consistent trend for dubbed birds to shift to the lower social positions in the flock. After dubbing the top ranking bird in all flocks was a non-dubbed female. In 6 of the 8 flocks the top two ranking members were non-dubbed chickens. Dubbed birds composed the lower half of the peck-order in Flocks 1, 2, 6 and 7. The length of the waiting period before being returned to its respective flock did not appear to effect social position. This is indicated by comparing Flocks 1 and 2 with Flocks 6 and 7. In the former 2 groups the dubbed birds were returned im-

Table III. Peck-orders in Flocks of White Rocks Prior to and After the Removal of the Combs and Wattles from Certain Members.

Flock[1]		Peck-orders
1	Pre-dubbing	A →B² →C →D →E² →F
	Post-dubbing	D →F →A* →C*
2	Pre-dubbing	A →B →C →D →E² →F
	Post-dubbing	A →C →D* →F* →B*
3	Pre-dubbing	A →B →C² →D →E →F
	Post-dubbing	B →D →A* →E* →F
4	Pre-dubbing	A →B →C →D →E →F
	Post-dubbing	A →F* →B* →C →D* →E
5	Pre-dubbing	A →B →C →D →E →F
	Post-dubbing	B →A* →F →D →C* →E*
6	Pre-dubbing	A →B →C →D →E →F
	Post-dubbing	C →A →E →D* →B* →F*
7	Pre-dubbing	A →B →C →D →E →F²
	Post-dubbing	B →D →C* →A* →E*
8	Pre-dubbing	A →B →C →D →E →F
	Post-dubbing	A →C →B* →E →F* →D*

1. Dubbed and non-dubbed females in Flocks 1 through 4 were placed in neutral pens 3 weeks post-dubbing while in Flocks 5 through 8 this was done immediately after dubbing.
2. Died during the course of the experiment.
* Dubbed birds.

mediately after dubbing, while in the latter 2 they were not returned until 3 weeks later. The lowest social positions in each of these 4 flocks were occupied by dubbed females.

Discussion

Data obtained in these experiments indicate that the removal of the combs and wattles from pullets influenced their social position when they were intermingled in flocks with non-dubbed chickens. Observations of peck-right relationships in large flocks provided evidence which indicated that the dubbed pullets were subordinate to non-dubbed females. After these large flocks had been housed for 107 days not a single encounter about the combination feeder-waterer was won by a dubbed chicken over a non-dubbed bird during any observation period. This indicated further a social stratification within each flock with the dubbed birds ranking in the lower social positions.

Analysis of the data in Experiment I was made using the technique of asymptotic simultaneous confidence intervals. This method of analysis was well adapted to this type of data and should prove very helpful to investigators connected with the behavioural sciences.

The experiment involving small flocks was undertaken to determine if this stratification between dubbed and non-dubbed birds would also be present in small flocks where relationships between birds are based, to a greater degree, on individual recognition. It was observed that after birds were dubbed and returned to their respective flocks, undubbed birds did not recognise their previous penmates. This resulted in the formation of new peck-orders. These findings substantiate those of Guhl & Ortman (1953) who reported the importance of the comb in recognition among chickens and confirm the findings of Hale (1956) who reported a loss of recognition when birds were dubbed.

After the small flocks were reorganized the lower half of the social order was composed largely of dubbed birds. Collias (1943) reported that comb size was a major factor for success in

initial encounters between birds. There may have been this psychological advantage for undubbed birds when the dubbed birds were returned to their original flocks. It is possible that the dubbed birds could recognise their former pen-mates in pens where birds were returned immediately after the dubbing operation. They might, however, have trouble maintaining their social position during a revolt because of their recent operation.

Guhl (1953) cites Schjelderup-Ebbe (1935) as having shown that separation for 2 or 3 weeks resulted in failure of birds to recognise former penmates. Perhaps the psychological effect of comb size was the reason that dubbed birds, which were not returned to their respective pens until 3 weeks post-dubbing, did not win back their original positions in the peck-order.

Hale (1956) reported that in social interactions between breeds of chickens behavioural responses were based on breed recognition rather than individual recognition. Individuals placed in flocks of another breed were frequently accepted as dominant birds without challenge if members of that breed had previously defeated or dominated members of the other breed in that flock. In large flocks where individual recognition of all birds would be difficult it may be possible that there would be a combination of individual and group recognition. If, after losing several initial encounters, dubbed birds became stratified into the lower social positions, the groups of dubbed and undubbed birds could have responded in a manner similar to the breeds in Hale's (1956) experiment.

Summary and Conclusion

A study was conducted to determine the influence of dubbing on the social behaviour of chickens maintained in inter-mingled flocks with undubbed birds. Analysis of the data obtained were made using the technique of asymptotic confidence intervals for the probabilities of multinominal distribution. This was the first time this method has been used on biological data.

A definite effect was observed in dominant-subordinate relationships as a result of dubbing. When intermingled in large flocks with undubbed chickens, dubbed birds were subordinate to non-dubbed chickens. After flock organization became more complete a definite stratification became more apparent with the dubbed birds ranking in the lower social positions. This same effect was also observed within small flocks.

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TOWARDS DEFINITION OF THE STIMULUS SITUATION FOR THE APPROACH RESPONSE IN THE DOMESTIC CHICK

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It is now generally established that birds of several species will follow a moving object during a period shortly after hatching. Hinde, Thorpe & Vince (1956) have shown for example that young hand-raised Moorhens and Coots will follow a variety of objects and can generalize to objects other than those used in earlier trials, provided that they are in motion. Nice (1953) working with coots, ducklings and Franklin gulls found that the movements of the hand while sketching were not sufficient to establish imprinting. On the other hand, Fabricius (1951) has maintained that movements of parts of a body in relation to each other, as in vertebrate motion, are more important for the first release of the following reaction than any properties of shape or movement of the body as a whole. Weidmann (1958) has noted that some Mallard ducklings will approach a slow moving human being or a seated person if the upper part of the body is swayed from side to side.

These observations suggest that some further experiments might be designed to define the properties of the stimulus situations which appear to evoke the initial movement of approach.

General Method

Eggs of the domestic fowl (Light Sussex × Brown Leghorn) from an attested breeder were hatched in an incubator. The chicks were ringed soon after hatching and placed in a covered box in a darkened drying compartment. Apart from the hands of the operator, one of which shielded the head, the chicks would very probably have had no visual experience of a moving object and certainly no sustained experience of a moving object until placed in the test run. This was a rectangular enclosure 10 ft. × 2·5 ft. × 2 ft. high, covered on all vertical sides by opaque brown paper and on the top by a fine muslin cover and lighted and heated by five radiant heat lamps. It was built in the department and is described by Salzen & Sluckin (1959). The room was in darkness. Chicks were placed at the centre of the run and facing at right angles to the longitudinal axis. Testing was done as far as

possible between the ages of 16 and 26 hours; but since there was no way of knowing the precise time of hatching for each chick, there may have been some variations slightly beyond these limits. This would not have affected any one experimental condition consistently. In taking the chicks from the darkened box, the experimenter shielded himself and seized the chick gently, placing the palm of the hand over the head. Apart from the endeavour to ensure as far as possible an equal number of each sex for experimental groups, no other factors of selection could have been involved.

Every precaution was taken to ensure silence in the experimental room which was insulated, though not perfectly, against sound. There is no reason for believing that extraneous noises from outside effected one experimental condition more than another. No intake of food or water was permitted until experiments were completed so that the results cannot reflect reinforcement.

Experiment 1. Comparison of the Effectiveness of a Rotating Black and White Disc with an Identical Disc Moving Towards and Away from the Chick

Procedure

In a preliminary study, a black sector (45°) was painted in indian ink on a disc of white Bristol Board which was placed at the end of the run and rotated clockwise at one revolution per 1·5 seconds by a small and, as far as could be determined, silent electric motor. Of seven chicks from one batch, five moved steadily towards the disc, going virtually the whole distance within the five minutes allowed. Four ceased to emit the loud, high pitched ('fear') chirps and changed to softer chirps of lower pitch. In three cases pecking in front of the disc accompanied the soft chirps. Approximately 45 minutes later, the same five chicks moved towards a 12-in. disc with two black (45°) sectors, rotating at the same speed. In a subsequent experiment, of three chicks from another batch, two moved to a rotating disc bearing two sectors. About twenty minutes later all three moved to a disc with one black sector and remained fixated before the disc, chirping softly.

In the main experiment there were two conditions, presented separately. In one the rotating black sector on white was placed at one end of the run, and the chick, facing at right angles to the sector, at the centre of the run. There was no other variable stimulus in the run and the chick could thus approach and retreat an equal distance from the sector. In the other condition, an identical disc was fastened to the base of a white, steel cylinder and hung from an overhead cableway. The disc, with the upper edge of the black sector horizontal at 9 o'clock, could thus be moved repeatedly up to the chick, facing at right angles in the centre of the run, and away. This disc did not rotate, and was the only variable stimulus in the uniform run. In view of the considerable variation noted in the department between batches in approach and following responses, an equal number of chicks from each batch were presented with the one stimulus situation for five minutes. The disc moving to and fro was moved slowly up to and as close to the chick as possible without touching. It was then moved away more briskly to a distance of about 18 in. and returned slowly. This was repeated until the chick began to follow, when it was maintained a few inches in front of the chick. The procedure was continued until the chick had followed as far as the disc would go in one direction or five minutes had elapsed. The distance moved towards both stimulus objects was noted from gradations marked along the side of the run.

The experimenter sat at the side of the run, slightly behind the point at which the chick was inserted so that in all cases scored as an approach, the chick moved away from the experimenter. A response was scored as an approach if the chick moved consistently towards the stimulus without turning away. All but three of the 17 which approached the rotating disc, lowered the head before the disc and emitted soft, low chirps.

It should be noted that it was impossible to prevent some lateral and vertical movement of the disc moving to and fro and that some slight noise at times accompanied the movement of the cableway. Despite this, the disc rotating in one plane was more frequently associated with movements of approach.

Results

(1) Comparing the assessment of whether the chick was judged to approach or not, the accompanying table indicates a definite superiority in favour of the rotating disc. Four separate batches of chicks were involved.

Approach to	No	Yes	Totals
Retreating disc	17	7	24
Rotating disc	7	17	24
Totals	24	24	48

χ^2 (corrected for 2×2 table) = 6·75, df=1. Sig. at ·01 level.

(2) The 17 chicks which approached the disc rotating in one plane covered 123 gradations (average, 7·2). The seven chicks which approached the disc moving away from them, covered a total of 30 gradations (average, 4·3). While the situations are not entirely comparable, the difference in the distances covered as tested by the Mann-Whitney U Test was significant at the ·02 level.

(3) The average time of first movement for the 17 chicks approaching the rotating disc was 71 secs. The average for the seven chicks approaching the retreating disc was 100 secs. The difference between the two groups, as tested by the U test, is not significant (p > ·10).

(4) There were an equal number of each sex in both experimental groups. Of the 17 chicks which approached the rotating disc, nine were males and eight were females. Of the seven which approached the disc moving to and fro, four were females and three were males.

Experiment 2. Rotating Black and White Disc Compared with Rotating White Disc of same Diameter (12-in.)—both centres 9 in. above the floor
Procedure

This experiment involved 40 chicks from four batches, and was carried out to cover the possibility that some noise or draughts associated with rotation may have contributed to the results in Experiment 1. There were two experimental conditions. Equal numbers of chicks from each batch were allotted to each condition. Twenty chicks were first placed individually at the centre of the run with a plain white disc of 12-in. diameter at one end and the other twenty were similarly presented first with the black sector on the white disc rotating at the same speed in the same position and for the same interval, five minutes. Two different chicks were exposed successively to one stimulus and then

to the other so that within a period of approximately 12 to 15 minutes, each chick was presented with the other stimulus situation.

Results

No case was recorded of a chick moving towards and remaining fixated in front of the rotating, plain white disc. Of the 40 responses to the white disc in the two groups, 16 involved apparently haphazard running to and fro, 6 were movements solely away from the white disc and 18 chicks did not move at all. Of the 40 possible responses to the rotating black sector, 25 involved clear and consistent movements of approach with final fixation, three, movements away from the disc, four, movements to and fro and eight, no movement at all. Of the 25 which approached the rotating black sector, twelve approached on the first presentation and thirteen on the second. Both of these results are significant in terms of Fisher's Exact Probability Test (p= ·000022 and ·0000064, respectively).

These results suggested that approach to and fixation before the black and white disc were associated with some form of intermittent stimulation and led to the next experiment.

Experiment 3. Rotating Black Sector on White Disc Compared with Flickering on/off Patch of light Diameter 6 in. and Centre 6 in. from the Floor

Procedure

This experiment involved 60 chicks from five batches, arranged in two groups of 30, each with an equal number of both sexes. The two experimental conditions were presented separately. The rotating sector was the same as used in the other experiments. The flashing patch of light was achieved by placing a D.C. car headlamp bulb (72 watts) in a cardboard box, painted black on the outside with a circular hole cut in the front. A sheet of frosted glass was placed over the hole on the inside of the box and a sheet of thin black carbon paper over the outside. This gave a dull, off-white patch (6-in. diameter) of light intensity approximately ·5 foot-candles as measured by a barrier layer cell at the surface. The light was 'on' for $\frac{1}{4}$ sec. and 'off' for $\frac{1}{4}$ sec. The timing apparatus was placed in a distant room to avoid noise. Each chick was presented first with one stimulus for five minutes and approximately 15 minutes later with the other, for the same period. Each sequence involved 30 chicks. As before, the chick was placed at the centre of the run facing at right angles to the

stimulus. With no other variable stimulus in the run, the chick was free to retreat from or approach the one stimulus by an equal distance.

Results

(1) A very poor response was recorded from two batches; but the accompanying table indicates the assessment of whether or not the chick was judged to approach and fixate. Equal numbers from each batch were allotted to each experimental condition.

Approach and fixation	To flickering patch		To rotating sector	
	Yes	No	Yes	No
On first presentation	11 (6M.5F)	19	10 (6M.4F)	20
On second	14 (6M.8F)	16	11 (6M.5F)	19

Viewed as a stimulus object, the flickering patch of light is not significantly better than the rotating black and white disc, either on first or second presentation. (χ^2 contingency tests for both first and second presentations are not significant.)

(2) Of ten chicks which approached both stimuli, five approached the flickering light and five the rotating disc on first presentation. In both of these groups two were females and three were males.

(3) The difference between the distances covered by those chicks which approached and fixated before the flickering patch of light (average, 6·6 gradations) and those approaching the rotating sector (average, 7·3 gradations) on first presentation, are not significant in terms of the U Test (p>·10). The differences on second presentation are also not significant (p>·10).

(4) The difference between the times of first movement are again not significant, as determined by the U Test, for both first and second presentations (p>·10, in each case).

Conclusions

The findings are consistent with the hypothesis that the visual stimuli effective in evoking initial approach and fixation in this species include an intermittent element. Further investigation might establish optimum conditions of size, frequency and intensity and in so far as movement is involved in the stimulus situation, the relative importance of vertical and lateral components. The appreciable variations in frequency of re-

sponse between different batches also calls for investigation.

Summary

1. From a comparison with two groups of 24 newly-hatched domestic chicks, a black sector on a white background, rotating slowly in one plane, was found to be a significantly more effective stimulus in inducing initial approach than a similar object moving away from the chick, but not rotating. Both stimuli were presented separately to equal numbers of chicks from the same batches.

2. From a study with 60 chicks, in which two groups of 30 were exposed first to one stimulus then the other, a flickering patch of light 6-in. in diameter was also found to be an effective stimulus in inducing approach, but not significantly better than a rotating black sector on a white background. Both stimuli were capable of eliciting approach with lowering of the head, pecking, and low, soft chirping. All the chicks which approached these stimuli remained fixated either before the stimuli or in the near vicinity. A chick might peck slightly to one side, but would always reorientate towards the stimulus.

3. In an experiment with 40 newly hatched chicks, not one fixated before a rotating, plain white disc. Twelve chicks of twenty, first presented with the rotating black sector, approached and fixated before it; but did not fixate before the rotating plain white disc. Thirteen of the twenty, first presented with the rotating plain white disc, did not fixate before it but subsequently did approach and fixate the black sector. A frequent response to the plain white disc was rapid running to and fro.

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THE HONEYBEE QUEEN AND HER ATTENDANTS

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Introduction

The worker bees forming a circle round the queen of a honeybee colony (*Apis mellifera* L.) have been studied from several different points of view. For example, Rösch (1925), Perepelova (1928), Taranov & Ivanova (1946), and Allen (1955) have recorded the ages of various groups of attendants, and observations have been made on the proportions of the attendants licking and examining the queen, and on changes in behaviour to the queen as swarming time approaches (Allen, 1956; 1957; 1959).

Another aspect of colony life in which the attendants may play an important part is in the perception of the presence of the queen. Should the queen be killed or removed the whole colony can rapidly become aware of the loss, thus demonstrating one facet of what is known as "colony cohesion", or the functioning of a colony as a single unit. Various theories have been put forward to account for aspects of colony cohesion, of which perhaps the most notable are those of Gerstung (1891-1926) and Butler (1954), but for a better understanding of the mechanisms involved it seemed desirable to have further data concerning the behaviour of the queen's attendants. The problems which have been studied in the present work include the length of the visits paid by the attendants to the queen, and investigations of whether all the attendants tend to remain with the queen for as long as possible on each visit and of what relationship there is between age of attendant and length of visit. Linked up with this study records were made of factors connected with the feeding of the queen.

Methods

In order to have bees of known age in the colonies studied, groups of 100-110 newly emerged bees were taken from other colonies, marked on the thorax with a spot of distinctively coloured cellulose paint, and, after allowing to dry for about 30 minutes, were introduced into the observation hives. In Colony A (1954), which was used only for observations connected with the feeding of the queen, marked bees were introduced every fourth day from 17th May to 18th June, and in Colony C twice weekly from 28th March to 9th July. In Colony C an average of 12 bees in each group was given a second spot of paint on the thorax in addition to the group mark so that they could be recognized individually.

The approximate numbers of bees in the colonies were as follows:

Colony A. 18th May, 1954 .. 4,500 bees
5th July .. 10,000 bees
Colony C. April and early May, 1957
2,500 bees
2nd June .. 4,000 bees
18th June .. 7,000 bees
8th July .. 10,000 bees

Both colonies had one-year-old queens. Colony A swarmed on 7th July, 1954, and Colony C on 13th July, 1957.

The majority of observation periods lasted for one hour each day; in some cases they were longer or shorter, although never less than half an hour.

Results

1. Duration of Visits to the Queen

As the chief aim was to gain some information about the length of time which different bees spent with the queen, records were made of all visits to the queen of Colony C by individually marked bees in the periods when she was under observation between 11th April and 13th July, 1957. It was found that many bees lost contact with her when she moved, whereas others left voluntarily, and since it was felt that the so-called voluntary departures were of greater interest than the other type when considering the behaviour of the attendants only these records will be presented here.

A total of 196 individually marked bees were observed to examine the queen and leave voluntarily, but many of these paid her more than one visit. The duration of visits varied from more than half an hour to almost immediate departure and there were all gradations from an apparent desire to avoid the queen to a desire to stay with her, the latter being manifested both by the relatively long periods of examination and by the attendants following her when she moved.

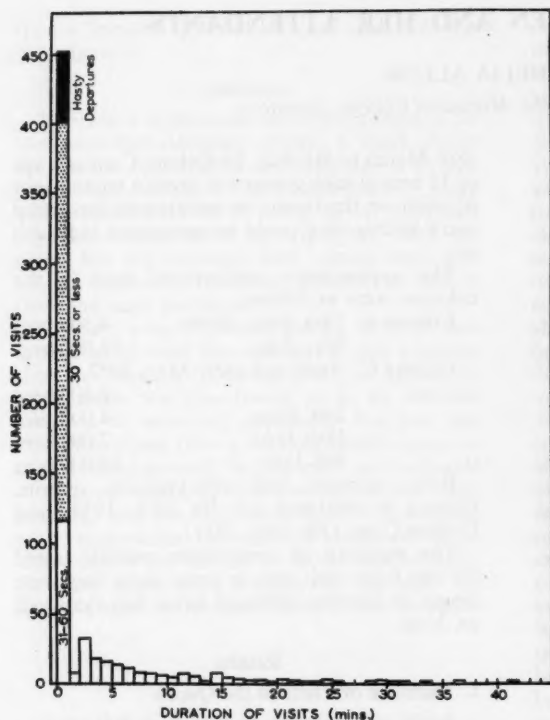In Fig. 1 the number and duration of visits are shown, the results being divided into periods

Fig. 1. Duration of visits to the queen by marked bees, Colony C.

lasting for one minute each. 451 visits (73 per cent. of the total) did not last longer than one minute and the number declined steadily as the duration increased: of the 625 recorded visits 87 were longer than five minutes, the longest being 41 minutes. The visits lasting for one minute or less can be arbitrarily divided into two groups: one consisting of 335 visits of up to 30 seconds and the other of 116 visits from 31 to 60 seconds in duration. The first group can be subdivided on the basis of behaviour because 50 of the 335 visits terminated rapidly, giving the impression that the bees were actively avoiding the queen, while on the remaining visits (including those lasting only a few seconds) the bees left the queen quietly and with none of the signs of alarm seen in the other group.

Many bees visited the queen more than once while under observation, since 196 individual bees made 625 visits; these repeated visits occurred in some cases over a period of several

days. Seven bees paid the queen ten or more visits. Each of these bees was recorded in at least three of four arbitrary time categories (Table I), and five of them made "hasty departures" on one or more occasions. The significance of the "hasty departure" is an interesting question; as the results in Table I indicate, the behaviour of any one bee was variable. Only 6 of the 196 observed bees were recorded solely under the heading of "hasty departure", while 36 bees made these and longer visits. This strongly suggests that this avoidance behaviour was spasmodic, and moreover among these 36 bees there was no apparent sequence in the lengths of visits, and the "hasty departure" category of visit occurred before, after or between the longer visits. None of these bees was observed continuously over a long period, however, and it is possible that if this had been done a recognizable pattern might have emerged.

2. The Relationship Between Age of Attendant and Duration of Visit

The ages of all the attendants discussed in the previous section were known and the results were examined to discover if they showed any evidence of a correlation between age and duration of visit. For the purpose of also ascertaining the relative frequency of appearance of different ages, the records for days

Table I. Number of Visits Paid by Seven Individual Bees

Symbol of bee	Time with queen				Total no. of visits
	>1 min.	½-1 min.	<½ min.	Hasty departure	
Gr$_{gr}$	6	1	3	0	10
Mn	4	3	2	1	10
Tp	0	1	8	2	11
Am	1	2	6	2	11
Tw	2	3	7	0	12
Pn	1	2	8	3	14
Gr$_w$	7	6	5	1	19

between 11th and 25th April have been omitted in order to consider only those days on which there were marked bees of all ages up to at least 28 days old in the colony.

Fig. 2. Ages of bees visiting the queen, Colony C.

3. Ages of Attendants Licking the Queen

While visiting the queen certain attendants licked her for periods of varying duration, interspersed with periods during which they examined her with their antennae. It is difficult to obtain accurate records of numbers of lickers of different ages because of the intermittent nature of the licking process on many occasions, and so it was decided simply to record the first licker of each colour group seen each day in Colonies A and C in order to determine the age-range. The results showed that attendants from one to thirty days old licked the queen, indicating that all ages could perform this duty.

4. Attendants Feeding the Queen

In addition to examining and licking the queen a few attendants fed her, and it was possible to record the age of every marked bee seen to supply food. In Colony A (1954) the observations commenced on 27th May, and in Colony C (1957) on 17th April. No *marked* bees were seen to feed the queen after 21st June in Colony A or after 24th June in Colony C, although observations were not terminated until the prime swarms left on 7th July and 13th July, respectively. (This absence of records of marked bees during the final two or three weeks is thought to have resulted from a decrease in the proportion of marked to unmarked bees following a progressive increase in colony size at this time, as well as from a decrease in the number of feeds—see Figs. 5A and 5B). Only feeds of longer than four seconds were considered since it is doubtful if any appreciable amount of food was supplied during shorter periods.

Sixty-one records were obtained from Colony A and 82 from Colony C; the results are given in Fig. 3, which shows that in both colonies the minimum age of food suppliers was 1 day, and that in Colony A the maximum recorded was 11 days whereas in Colony C it was 23 days. No marked bee was seen to feed the queen on the day of its emergence. The actual numbers recorded were greatest during the first week of

The visits were divided into two categories, namely those of longer than 30 seconds and those of 30 seconds or less, and the distribution of ages obtained in the two groups is shown in Fig. 2. There seems to be no clear evidence of a difference between the groups. There must have been a decline due to death in the number of marked bees in the colony as their age increased, and so there was a greater likelihood of observing young marked bees than older ones; the declining frequencies in Fig. 2 may therefore only represent the decreasing numbers of older marked bees present in the colony. If the attendants were a random sample of the whole population one would expect to find that the greatest number of marked attendants consisted of the youngest bees, since there were more of these available, but Fig. 2 shows that in both groups the actual numbers are not as high as would be expected on this assumption, particularly in the case of the visits lasting for more than ½-minute. There was therefore apparently a tendency for the queen to receive relatively fewer visits from the youngest bees than from the somewhat older bees.

Fig. 3. Ages of bees feeding the queen.

adult life and reached a peak on about the third or fourth day in both colonies.

When comparing the frequencies with which different ages were represented it would be theoretically correct to make an adjustment for the number of hours of observation during which it was possible to observe marked bees of each different age, since the duration of observation periods varied to some extent from day to day, but it was found that the distribution of the results remained virtually unchanged after such an adjustment (Allen, 1958). The declines following the peaks seen in Fig. 3 were partly due to the fall in total numbers of older marked bees mentioned earlier but an accurate correction for this factor cannot be made. However, in Colony C it is possible to compare the age-distribution of the feeders with that of the attendants. For this purpose no records from dates before 25th April, 1957, were considered, in order to cover comparable periods in the two groups; it can be seen in Fig. 3 that this adjustment makes little difference to the age-distribution of the feeders. Comparing Fig. 2 (attendants) with Fig. 3 (food suppliers) it is evident that there were relatively more young bees among the food suppliers than among the attendants. (Taking 8 days as an arbitrary dividing point, the relative numbers of bees less than this age were compared in the two

groups by means of a χ^2 test with the numbers 8 days old or over; $\chi^2 = 35 \cdot 267$, p $< \cdot 001$, and therefore it is very highly probable that the difference between the ages of attendants and food suppliers was not due to chance alone) .

In order to investigate the possibility of there being a change in the age of the food suppliers as the summer advanced and swarming time approached, the mean and maximum ages were plotted against date. In Colony A there was evidence of a drop in age following the formation of the first queen cup on 9th June (Allen, 1955) but no marked bees were seen to feed the queen in the fortnight preceding swarming, with the result that the numbers in the second period are rather small. In Colony C no marked bees fed the queen while under observation during the period of swarm preparations and no comparison of the two periods could be made.

It is widely thought (e.g. Ribbands, 1953, p. 58) that the food given to the queen is very similar to the larval food which is secreted by the pharyngeal glands of the workers. Since the development of these glands is to some extent related to age—they are not fully developed at emergence and tend to retrogress in later life (Soudek, 1927)—it would be expected that the ages of the bees feeding the larvae and of those feeding the queen would be similar. Rösch (1925) recorded the ages of 48 marked bees feeding larvae and found an age range from 3-13 days, and Perepelova (1928) obtained a very similar age-range for 87 marked bees (3-16 days); Lindauer (1952) made far more extensive observations on more than 470 nurse bees and found that they varied in age between 1 and 30 days, with a peak in numbers towards the end of the first week of adult life. Lindauer's results indicate that numbers decline to low levels after about the eighteenth day of life, which is broadly in keeping with the maxima of Rösch and Perepelova, if allowance is made for their much smaller number of records.

Comparing Lindauer's figures for the ages of nurse bees with those obtained here for the ages of bees feeding the queen it is found that both the ranges and the peaks of the curves are approximately similar, although the peak of the queen-feeding curve is situated about the fourth day of life and that of the larvae-feeding curve at about the end of the first week.

Rösch and Perepelova both suggested that the younger larvae were fed by bees of more than five days old, but Lindauer's figures show no such differentiation. Lindauer also studied the

pharyngeal glands at different ages and found that there was considerable variability in the degree of development. The available evidence indicates that there is no absolute minimum age limit for the production of brood food and therefore there is no necessity to assume that the youngest bees provide the queen with a type of food different from that given by the older bees. Nevertheless, if there were such a difference it might be reflected in the length of feeds, since the consistency of the food might vary and consequently take a longer or shorter time for the queen to receive. This relationship between age of feeder and duration of feeds was therefore investigated and the results are given in Fig. 4. where the duration of each feed given by the marked bees in both colonies is plotted against age and the mean values for various ages are shown. The means were calculated from groups of 15-23 records (mean number of records per group=20). It is clear that although the duration of feeds varied from a few seconds to rather more than 2½ minutes the mean value remained approximately constant in relation to age (overall mean of individual figures=44 seconds).

Perepelova (1928) in her work on the ages of attendants feeding the queen recorded no feeder under the age of six days and the peak of her curve of frequencies occurred in the earlier part of the second week of adult life, and not in the first week as found here. A further point of difference is that Perepelova's mean for the duration of feeds was almost four times as great as that for the present colonies (173 seconds as compared with 44 seconds). Both sets of results, however, agree in showing no regular changes with age. The differences might be due to one or more of several factors, including time of year and differences in colony size and strains of bees, but Perepelova does not give details on any of these points. As regards colony size, Colonies A and C were probably both larger than average observation hive colonies but nevertheless they were small compared with most of those kept in normal hives, and the egg-production of the queen was correspondingly low—possibly one-third of the normal rate at the peak of the egg-laying cycle. From this one would surmise that the queen's intake of food might have been reduced proportionately. As will be seen in the next section, both the number and duration of feeds appeared to increase concurrently with egg-production, and if Perepelova's colony was of normal size both quantities might therefore have been greater than found here. It does, however, seem a little unlikely that this factor alone would have resulted in so large an increase in duration of feeds, especially as Perepelova states that the normal rate of feeding was 2-3 feeds per hour at the queen's egg-laying peak, and this was rather lower than that in Colony C.

5. The Feeding of the Queen in Relation to Her Egg-production and to the Approach of Swarming Time

Preliminary observations have been published on the feeding of the queen in Colony A and on her output of eggs (Allen, 1955), and it was found that the egg-laying rate remained fairly steady, apart from an indication of a fall in the few days preceding swarming. The number of feeds received per hour was, however, markedly reduced during
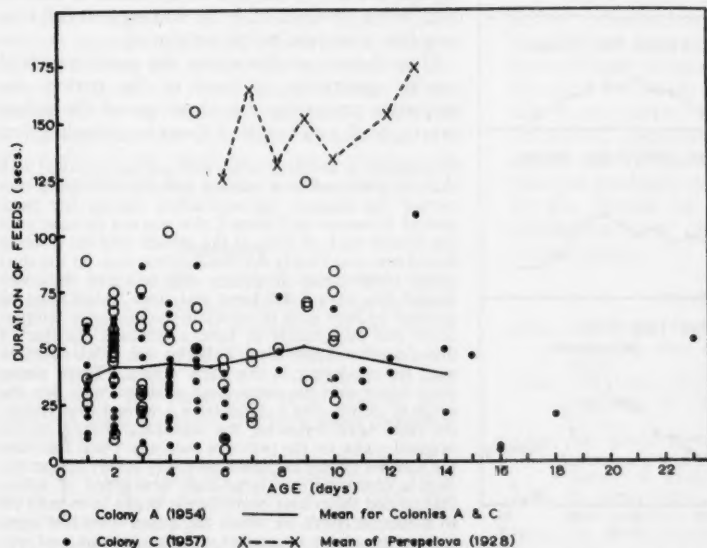
Fig. 4. Duration of feeds given to the queen by bees of different ages.

the latter half of June and in early July, when no feeds were observed.

Day-to-day observations of the queen in Colony C were made in order to obtain more comprehensive data on the same subject and the duration of all feeds (whether by marked or unmarked bees) was noted; Fig. 5 presents the means calulated from consecutive 4-day groups of results. The original figures as well as details of the observation periods are given by Allen (1958).

Apart from a possible peak in the third week of April the egg-laying rate (Fig. 5A) remained low until a steady rise started at the beginning of June. The fall just before swarming which was found in 1954 was again apparent at the beginning of July. The general form of this curve follows the same course as the corresponding part of the annual brood curve in a full sized colony (Nolan, 1925; Allen & Jeffree, 1956) and this indicates that apart from the minor fluctuations already discussed the queen in Colony C was behaving in a normal manner in respect of egg-production.*

The number of feeds (Fig. 5B) fluctuated but there is some indication of a rise coinciding with an increase in egg-production at the beginning of June. From a peak in the second and third weeks of June there followed a decrease which continued until the swarm left on 13th July. The mean duration of individual feeds (Fig. 5C) had a tendency to rise throughout the period studied and also reached a peak in the third week of June, after which there was again a steady decrease until the swarm left. The mean duration of all feeds was 42 seconds, as compared with 47 seconds in Colony A, thus showing a close agreement between the two sets of results.

Neither the number nor the duration of feeds, considered alone, is an index of the amount of food which the queen receives. The total duration of feeds per hour, which is in effect a combination of these two factors, may be a closer approximation to this. Fig. 5D gives these values and shows that the picture is not basically altered from that seen in Figs. 5B and 5C, although the rise in the first three weeks of June is even more obvious, as is the subsequent fall to a very low level just before swarming.

Thus there is evidence that the queen received smaller quantities of food in the period immediately preceding the departure of the prime swarm, both as a result of fewer bees feeding her
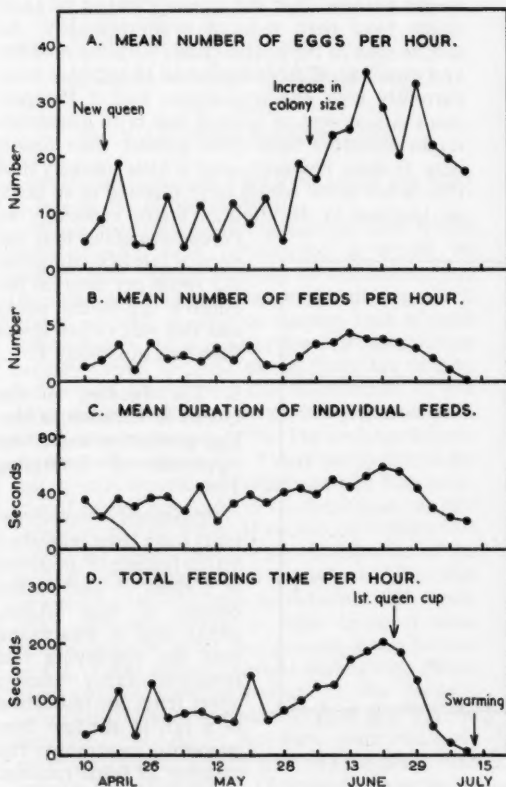
Fig. 5. Feeding and egg-laying rates of the queen, Colony C.

*Conditions in an observation hive are not identical with those in a normal hive because limitations of space may curtail the queen's egg-production during her peak period. However, in Colony C this was not the case until the fourth week of June at the earliest and the limiting factor here was clearly the small colony size. At the start of the observations the colony only occupied one comb (rather less than 3,000 bees) and since brood must be covered by bees both to maintain the necessary temperature and presumably to have a sufficient number of nurse bees to supply food, only this one comb could be used for egg-laying in the early stages. (There seems little doubt that the early small peak in April was the result of introducing a queen from a normal sized colony on 16th April following the accidental killing of the original queen on the previous evening). Until 2nd June the number of bees increased only very slowly but on this date a comparatively large-scale emergence of adults started and the colony immediately began to expand on to a second comb, in which the queen then laid eggs. Colony expansion proceeded rapidly after this, and was paralleled by an increase in egg-production for at least the next three weeks.

and because of the decrease in the mean duration of feeds. The beginning of the pronounced drop in the amount of food received coincided with the formation of the first queen cup (Fig. 5D).

Conclusions

There has been a widespread belief in the past that the organization of the honeybee colony was centred on its queen, and in so far as the queen is the source of eggs and therefore of new bees for the colony this is true. It is evident, however, that the colony functions as a unit and that queen and workers are mutually dependent on one another. For example, the amount of food received by the queen varies according to the seasonal development of the colony and this in turn presumably influences egg production, which has been shown here to follow a similar course to the queen's food intake. The workers feeding the queen might all have possessed active pharyngeal glands and could consequently have fed the queen on their secretion. If this was the case then any factor affecting the degree of activity of these glands (e.g. pollen intake) is likely to have had a direct effect on the nutrition of the queen.

The queen does not exert an invariable attraction over all the workers, since they frequently showed little interest in her and stayed with her for only a few seconds while a number left with the utmost haste. On the other hand, some workers made considerable efforts not to become separated from her when she moved over the combs. Perhaps the most notable feature of the results, however, was the variability exhibited by individuals, and this may indicate that the behaviour of the workers is very plastic and closely related to the needs of the colony at the time.

Summary

1. 196 individually marked bees were seen to examine the queen and the durations of their visits were recorded. All gradations of behaviour varying from an apparent desire to avoid the queen to a strong desire to remain with her were found. The behaviour of individual bees varied on different visits, but no regular sequence could be found.

2. More than half the total number of visits had a duration of 30 seconds or less while the remainder were longer than 30 seconds, with a maximum of 41 minutes. About 8 per cent. of the visits were very short and terminated abruptly

and with signs of alarm, but no reason for this type of behaviour was apparent.

3. The ages of the attendants varied from a few hours to 52 days but no obvious difference in age-distribution between the attendants remaining for very short periods and those remaining for longer was found. In relation to the total numbers of marked bees of different ages present the proportion of workers acting as attendants showed no systematic variations, although in a colony where the average age was low (as in summer) the actual numbers of young attendants would apparently greatly exceed those of the older ones.

4. The age-range of attendants licking the queen was similar to the overall age-range of the attendants.

5. The ages of the attendants feeding the queen in two colonies ranged from 1 day to 23 days. No bee was seen to feed the queen during the day of its emergence, although the greatest numbers recorded occurred in the next few days of adult life. No systematic changes were apparent in the duration of feeds given by bees of different ages, the mean value for two colonies being 44 seconds. These results are discussed in relation to the work of other authors on the period of activity of the pharyngeal glands.

6. Observations made during the season of active brood-rearing up to swarming time (April-July) showed that the number and duration of feeds supplied to the queen tended to rise until 2-3 weeks before the swarm left but in this latter period both number and duration decreased, approximately from the time that queen cell formation started. Thus the queen received gradually diminishing quantities of food in the period of swarm preparation. Egg-production over the same period showed a similar trend.

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ANALYSES OF THE BEHAVIOUR OF COMMENSALS IN HOST-FACTOR.
1. A HESIONED POLYCHAETE AND A PINNOTHERID CRAB

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Introduction

On the whole, very little is known about the behavioural processes that enable animals which live in symbiosis with others to find their partners (Davenport, 1955, etc.). The responses of certain polychaete commensals to their hosts have been investigated and evidence presented that they are attracted to their hosts over considerable distances by chemical factors, which, though specific, are as yet unidentified. For such specifically acting agents we have used the generic term *host-factor*. Responses have been investigated with the use of a Y-tube olfactometer (Davenport, 1950) and a latex choice-apparatus (Bartel & Davenport, 1956). With such apparatus one may easily determine whether or not certain commensals find their host by chemical recognition, but one cannot readily identify any changes in behaviour brought about by the presence of host-factor. Some sort of apparatus was necessary which would enable us to identify and analyse in detail any such changes, particularly in those animals in which one subjectively can observe no clear-cut, overt "searching activity." Many common species of this type (e.g. the facultative commensal polychaete *Podarke pugettensis* Johnson) can be demonstrated, by statistical analysis of distribution data in the latex apparatus, to aggregate on the host, as a result of their response to a specifically-acting host-factor (Hickok & Davenport, 1957). But we needed to have at hand apparatus that would allow us to test critically the behaviour of certain species of which one could collect only a few individuals and that at the same time would give us more precise answers to the questions: Just what does host-factor make the commensal do? Is the behaviour of the commensal different in host-factor than out of it? Does its behaviour change as it moves across gradients? Can any such changes in behaviour be identified as taxes or kineses according to classical definitions (Fraenkel & Gunn, 1940; Kennedy, 1945)? Do any such changes have the effect of bringing the commensal closer to the source of host factor?

Apparatus, Methods and Material

An olfactometer developed by Varley & Edwards (1953) for studying host-finding behaviour in parasitic insects was found to be adaptable to our needs. Our adaptation consists of an hexagonal trough (Fig. 1) 17 in. × 8¾ in. × ¼ in. in transparent plexiglass. This trough was made by cutting a hole of the above dimensions in a plexiglass sheet and cementing it to a whole sheet. Sea-water was introduced through a
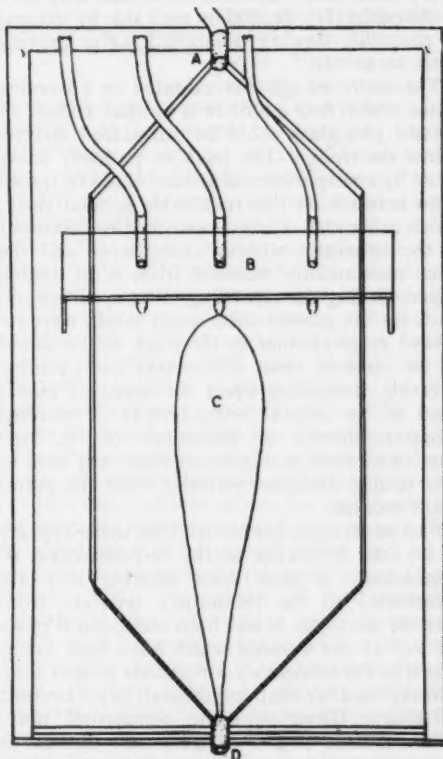
Fig. 1. Plexiglass test apparatus. A, inlet. B, plastic bridge with three pipettes. C, critical area. D, outlet.

tube (A) at one end of the trough. At (B) three streams of sea-water were introduced from drip-tubes set in a plastic bridge across the width of the trough. Non-circulating aerated sea-water was siphoned to the three tubes from three 3000-ml. beakers, which were maintained in a large aquarium water-bath at the temperature of the laboratory system (16·5°-18·0° at Santa Barbara; 19·0°-20·5° at Woods Hole). The flow from the drip-tubes was regulated by screw clamps. The middle beaker was used as a test chamber into which hosts and fluorescein (10⁻⁵ by weight) were placed. The inert dye has no effect on the behaviour of experimental animals, and it made the central stream carrying effective agents clearly discernible to the observer. In the very slow current flowing from one end of the trough to the other, this labelled experimental or central stream formed a spindle-shaped area (the critical area—C) from the central drip-tube to the outlet (D), limited on each side by streams of smoothly flowing unlabelled water moving at the same rate.

The entire trough was mounted on a wooden frame which had a slot in it so that a sheet of ground plexiglass could be introduced directly below the trough. The track or pathway being made by an experimental animal could be traced from beneath on this removable ground sheet, which at the end of a test run could be taken out of the apparatus without disturbance and the track permanently recorded from it on tracing paper. During the recording of an experimental track on the ground sheet small marks were recorded perpendicular to the track at the sound of an audible timer (10-second or 1-minute intervals, depending upon the speed of movement of the animal being tested). A wheeled distance-indicator or planimeter of the type used to measure distances on maps was used in determining distances travelled from the permanent records.

Past experience has shown that there appears to be little difference in the responsiveness of commensals to host-factor whether they are maintained in the laboratory separate from hosts or on them. It has been our general practice not to use animals which have been maintained in the laboratory for periods greater than a week, for after that commensals often become refractory. However, some commensal polychaete species (e.g. *P. pugettensis*) appear to retain their sensitivity to host-factor for long periods when maintained in the laboratory on their echinoderm hosts.

Hosts were placed in the central beaker not less than 12 hours before an experiment in the belief that host-factor might accumulate. Fluorescein was added just prior to the first test. In a standard test, the drip tubes would then be turned on, the clearly delimited central labelled stream allowed to become stable and an experimental animal introduced at a randomly selected spot near the bottom of the trough. Commensals were always handled with the greatest care using a camel-hair brush.

At the Marine Station of the University of California, Santa Barbara, a brief exploratory series of tests were conducted to determine the practicality of the apparatus as a tool with which to discern the presence of constant and identifiable responses to chemical agents. For this series the facultative commensal polychaete *Podarke pugettensis* Johnson was used, since it had already been demonstrated to respond specifically to an attractant released by the host. The results of this exploratory series led us to carry out extensive and more detailed experiments at the Marine Biological Laboratory, Woods Hole, where we used the obligate commensal crab, *Pinnixa chaetopterana* Stimpson.

In what follows it will be seen that, partly by necessity and partly as a result of experimentation, our methods of statistically analysing the pathways described by the two species differed; their method of movement and their behaviour in host-factor differed. The nature of the movement of an experimental animal in the apparatus will always in part determine the method of analysing pathways, e.g. pathways made by lengthened animals (polychaetes) which generally progress in the direction of the head will be quite different from pathways described by foreshortened animals (crabs), which frequently move sideways. Likewise, for example, pathways of animals which show little change in the rate of random turning (klinokinesis) when immersed in an agent, but marked tactic responses when crossing interfaces or moving through steep gradients will differ markedly from those animals which show a marked change in rate of turning but little evidence of directed responses (taxes). One must fit his method of analysis as best he can to the nature of the pathways of the animal concerned.

The statistical methods mentioned in the analyses which follow are described in detail in Siegel (1956). We have not accepted as significant probabilities of >0·01.
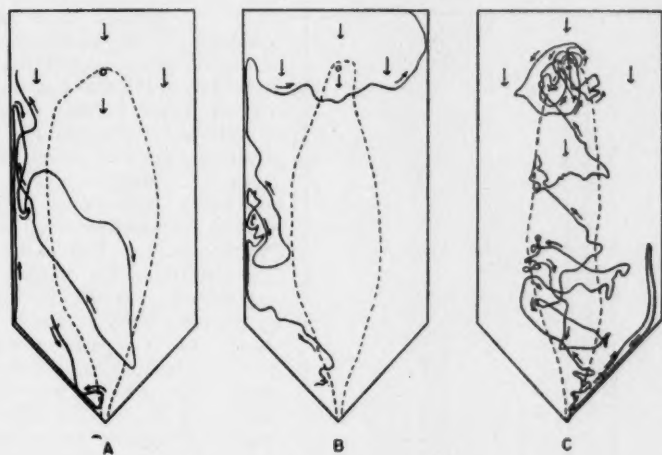
Fig. 2. Tracings of the tracks of *Podarke pugettensis*. A, a free-living worm, central area containing fluorescein-labelled host-factor. B, commensal worm, fluorescein only. C, commensal worm, fluorescein-labelled host-factor.

Experiments

The Hesionid Polychaete *Podarke pugettensis* Johnson

This worm is a facultative commensal which, on the Pacific Coast, inhabits mud-flats and wharf pilings and is commensal with two starfish, *Patiria miniata* and *Luidia foliolata*. Free-living and commensal worms behave quite differently toward host starfish. With the aid of the l atex choice-apparatus (Bartel & Davenport, 1956; Hickok & Davenport, 1957) it was shown that commensals are attracted strongly to hosts while free-living worms are not. Specificity studies were also made with the latex apparatus.

Tests with the plastic trough confirmed our earlier observations. Fig. 2 shows the tracks of free-living and commensal worms under different conditions. In A it can be seen that the random wanderings of a free-living worm appear to be unaffected by the presence of host-factor, as was also shown in the latex apparatus. In tests in which commensals encountered a central stream containing fluorescein alone (2B) there was no indication of any response to the fluorescein. In Fig. 2C, however, it can be seen that commensals are "behaviourally trapped" in a central fluorescein-labelled stream carrying water from an aquarium containing the host. Results of this sort are hereafter spoken of as positives.

Analysis of pathways for data with which to discern the presence of kineses or taxes was conducted on a series of 12 such positives in the exploratory tests. What do these data show?

Chemokinesis

We wished to know whether with this apparatus it is possible to demonstrate that when a commensal enters host-factor there is a change in linear velocity (orthokinetic response). Subjectively, one sometimes observes an increase in speed immediately after a *Podarke* encounters host-factor. However, it soon became clear that the apparatus is not too well suited to give data which enables one to discern by statistical means the presence of changes in rate of linear movement under the different conditions within it, for frequently a randomly introduced animal encounters the critical area so soon after introduction that one is not given enough data on its behaviour prior to entrance to the critical area to be able to make comparisons between this and its behaviour after entrance. Wishing to compare rate of linear movement for a similar number of intervals both prior to and immediately after entrance to host-factor, we selected eight positives from the series in which each worm had spent the first five one-minute intervals after entrance *entirely immersed* in host-factor (40 worm-intervals in host-factor) and in which each had also tarried five minutes before entrance (40 worm-intervals in sea-water). The distances per interval (speed) in the two media were compared using the Mann-Whitney U-test (Siegel, 1956). The results of this treatment indicate that the difference in speeds in the two media may occur by chance with a $P < 0.50$. The order of magnitude of the speeds in the two media did not appear to differ significantly. However, that the occasional subjective observation of an apparent increase in linear velocity after entrance to host-factor is valid can be seen in the plot of distance vs. time (Fig. 3) for the
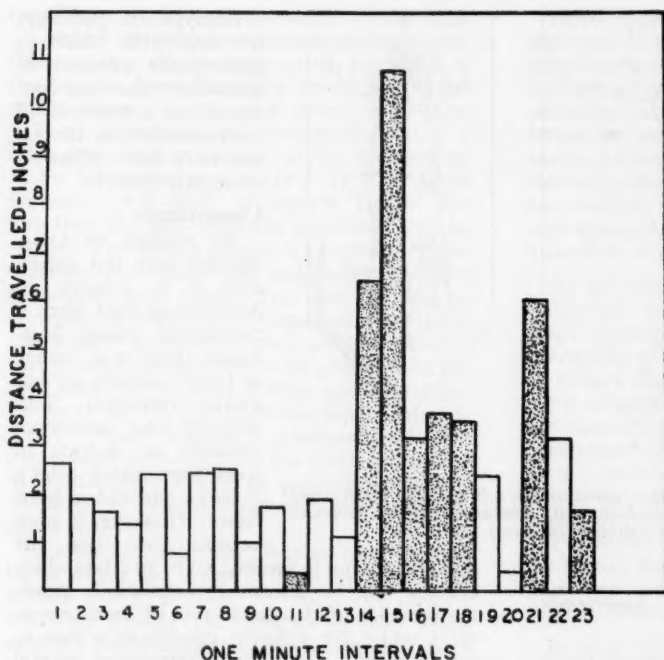
Fig. 3. Orthokinetic response of a single *P. pugettensis* in fluorescein-labelled host-factor. Unshaded bars indicate distance travelled per interval when outside host-factor, shaded when in host-factor.

track of one of the eight positives chosen for the statistical analysis above. At interval 11 the commensal was briefly exposed to host-factor at the constricted outlet of the trough and did not respond. But the velocity increased dramatically at intervals 14 and 15 after complete immersion in host-factor and then at 16, 17 and 18 dropped off towards the base rate. At interval 21 after a two-minute departure from host-factor the worm again speeded up after re-entrance.

One would wish to know whether upon a worm's entering host-factor a change in frequency of random turning (a klinokinetic response) can be discerned. An analysis was made of the frequency of change of direction for sections of the tracks inside and outside the host-factor stream. The number of changes in direction per cm. of track or per unit of time may be determined by the somewhat subjective method of Ullyott (1936) discussed in detail by Fraenkel & Gunn (1940). Fig. 4 indicates the method of making these determinations; each numbered angle indicates a change in direction of not less

than 5°, which angle was selected as the approximate minimum which could be so measured in the pathway of an animal which moves as does *P. pugettensis*. This method of determination is admittedly subjective because one is measuring movements which have been hand-recorded and hence subject to human error, and also because it obliges the experimentor to decide arbitrarily just what a change in direction is. The decision determines which turns are fractionated and which are not; arbitrarily in these studies a broad, sweeping curve was classified as a single change-of-direction. However, *P. pugettensis* rarely moves in this manner, changes in direction being generally followed by straight progressions of varying length. In our determinations, any turns occurring exactly at the interface of the host-factor stream were arbitrarily classified as having occurred outside the stream.
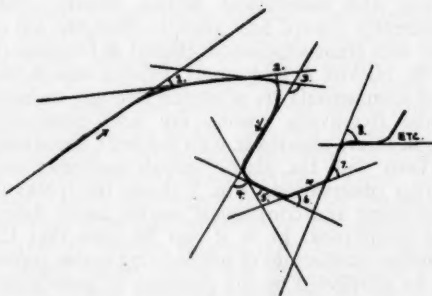
Fig. 4. Method of determining number of turns/cm. of track in *Podarke pugettensis*.

Twenty-five randomly selected one-minute intervals in host-factor showed a total of 198 turns as against a total of 113 turns for twenty-five randomly-selected intervals chosen from the tracks in sea-water prior to entrance. The frequencies of turning were computed from these

data and compared using the Wilcoxen Matched-Pairs Signed-Ranks Test (Siegel, 1956). Such treatment indicates that the two frequencies differ with a P<0·1 but >·05. Although our method of analysis is not well suited to discern minor differences which may be significant, these statistics support the conclusion drawn by the observer when he visually compares tracks made under the two conditions. There is certainly no dramatic change in the frequency of random turns such as we shall see occurring when *Pinnixa chaetopterana* enters host-factor.

Chemotaxis

One would wish to know whether with this apparatus it can be shown that contact with host-factor may result in directed responses (taxes). Data from twelve positives were analysed to determine whether on entrance to host-factor commensals turn towards the source more frequently than away from it. Turns toward the source are defined as turns with the sector enclosed by the 90° angle to right and left of the source. Turns away from the source are defined as those outside these sectors. The total number of turns towards the source from these tests was 83, while those away from the source totalled 26. The results were compared satistically using the Wilcoxen Test. The data differ significantly, the P being <0·005.

The same tests were analysed for data on

departures from the critical area. The total of turns toward the source on departure was 80, while the total away from the source was 30. This difference also appears to be significant (P<0·005).

Rheotaxis

One would wish to know the extent to which rheotaxis is important in determining the pathway in a positive such as Fig.2C. Subjective observation indicated that worms seem to have a tendency to head into the slow currents of the trough. In six tests in which no host-factor was present (as in Figs. 2B, 5C) net displacement was upstream in all. The worms appear to show a weak positive rheotaxis. Is this positive response to current strengthened by the presence of host-factor?

To answer this a series of 12 runs were made in which four positives of not less than 30-minutes duration (Fig.5A) were each immediately followed by a run of similar length in which the entire trough was filled with host-factor entering at the inlet (Fig. 1A) and at all three of the pipettes in the bridge (Fig.1B). We assumed that under these conditions no concentration gradient of host-factor exists in the trough. Each worm was then immediately given a third run of equal duration in the trough filled with labelled sea-water alone. Examination of Fig. 5 will show that under all three conditions there was no apparent difference in behaviour beyond the fact that in the positives (5A) the pathways of the animals became restricted to the critical area as they approached the source of host-factor, while in the two controls (5B, 5C) there was no such "behavioural trapping." In none of the runs of this series did the pathways of the worms appear any less devious when the animals were in a trough filled with host-factor (5B) than when they were in a trough filled with labelled sea-water alone (5C); there certainly appeared to
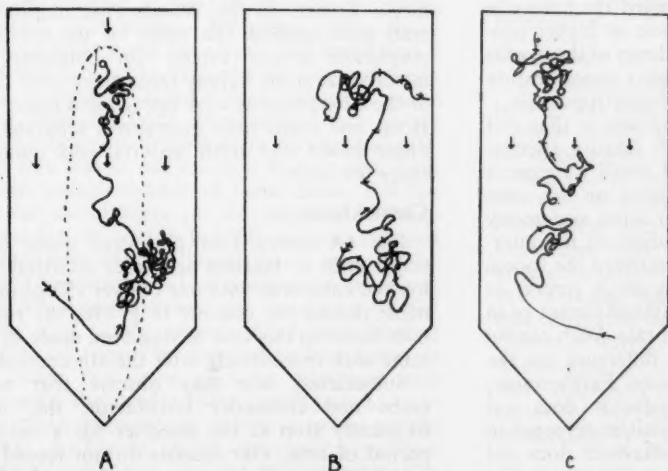

Fig. 5. Tracings of tracks of *Podarke pugettensis*. A, control positive. B, same worm, trough entirely filled with fluorescein-labelled host-factor. C, same worm, trough filled with fluroescein-labelled sea-water.

be no greater tendency to orientate the body parallel with the current.

Discussion

This exploratory series of tests with *Podarke* gave us clear indication that the plastic trough is a valuable tool with which to investigate the behaviour of aquatic animals in chemical gradients. The series was too brief to establish definitely the role of kineses and taxes in the responses to host-factor of *Podarke pugettensis*, but we nevertheless accrued data from which some preliminary generalities may be made.

Subjectively, one observes that the animals appear to be "physiologically trapped" in the host-factor stream and that they "climb up" it until they arrive in the vicinity of the source. The pathway described in Fig. 2C as the commensal approaches the source is characteristic of positives, the worm moving in a more and more limited field as the critical area becomes limited, passing out of the area and in again. Analysis of our data from positives for evidence of directed response indicates that the existence of such responses can be demonstrated with a very limited number of tests. It was shown that as the animals "climb up" the critical area they turn *toward the source* in the great majority of passages in and out through the interface between the central and side streams. The central stream carrying host-factor is without doubt diluted, particularly at its margins, as it passes down the trough. A turn toward the source is therefore a turn toward the side of higher concentration. The consistent tendency of the worms to turn in the direction of higher concentration can be defined as a positive chemotropotaxis.

The implication of this response is that this polychaete exhibits remarkable sensory discrimination in its discernment of small differences in the concentration of host-factor on two sides of its head. It must frequently sense asymmetry when it crosses the steep gradient at the interface; it turns more frequently toward the source whether the gradient is rising (as it enters) or falling (as it leaves). This behaviour cannot be in response to changes in current rate. With careful adjustment of flow-rates no difference on the two sides of the interface occurs. Furthermore, we have evidence that host-factor does not strengthen the worm's weak positive response to current, and therefore this behaviour does not appear to constitute a change in the response to current (rheotaxis) as the animal crosses the interface. If host-factor strengthened a positive

response to current, one would expect that absence of it on departure would in turn dampen this response. But the proportion of turns toward the source to those away from the source (83/26 vs. 80/30) is approximately the same whether the commensal is entering or leaving the host-factor stream, i.e. whether the animal is passing through a rising or a falling gradient. Although longer series of tests will be necessary to establish their importance in the behaviour of this worm, orthokinetic responses (changes in linear velocity) and klinokinetic responses (changes in rate of random turning) may be of some significance in the behaviour of this animal elicited by host-factor. One cannot discount the possibility that in nature, particularly in rapidly rising gradients close to the host, they may, along with the strong tropotaxis, effect "homing" on the host. Finally, the possibility that a klinotactic response (successive testing of concentration in time) may be of importance also cannot be discounted, but this phenomenon cannot be demonstrated under the conditions of our experiments.

The Pinnotherid Crab *Pinnixa chaetopterana* Stimpson.

During the summer of 1958 at Woods Hole it became possible to conduct extensive experiments with an obligate commensal, the crab *Pinnixa chaetopterana*, obtained from the tubes of the parchment worm, *Chaetopterus pergamentaceus* Cuvier. In the Woods Hole region the crab also inhabits the tubes of the terebellid *Amphitrite ornata* Verrill. On obtaining the material from the Supply Department, the tubes of the Chaetopterus were opened and discarded. Hosts and crabs were maintained separately in finger bowls and fresh material was obtained every few days.

Chemokinesis

Fig. 6A shows four pathways made by a single crab in labelled sea-water (control) and may be compared with one of over 150 positives made during the summer (Fig. 6B), the run in host-factor in this case having been made by the same crab immediately after the 4th control.

Subjectively one may observe that when crabs first encounter host-factor, they very frequently stop at the interface for a variable period of time. Our records do not record the length of time of this stopping; they only show ten-second intervals on the track from which linear velocity can be computed. For this reason
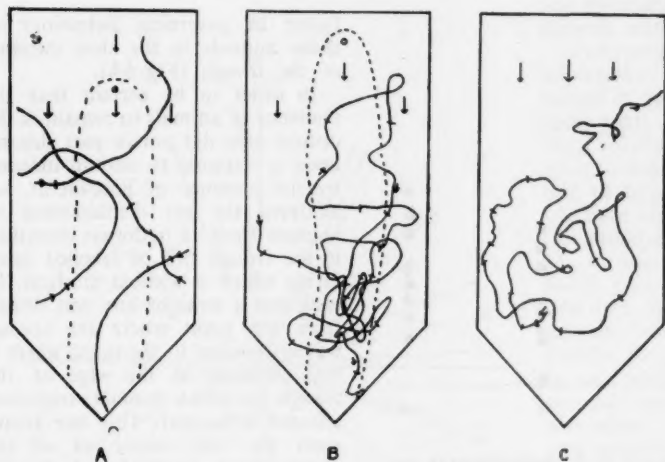
Fig. 6. Tracings of tracks of *Pinnixa chaetopterana*. A. four tracks made by a single crab, central stream carrying fluorescein labelled only. B, same crab run immediately against fluorescein-labelled host-factor. C, track of a crab in trough filled with fluorescein-labelled host-factor.

analysis to compare rate of linear motion before and after entering host-factor would not give valid results. There is, however, quite obviously no increase in linear velocity; indeed the cessation of linear movement on encountering host-factor may be defined as an akinesis.

When crabs move into host-factor there is a marked increase in the rate of random turning ("direct" klinokinesis—Kennedy, 1945). Often turning movements are so fine and rapid that it is difficult to make accurate trackings of them. This is because animals very frequently make very little net body displacement but simply pivot repeatedly around their dorsiventral axis. For this reason our tracings show a reduction of the actual number of turns made. But the general deviousness of the pathway in host-factor can be seen in Fig. 6B, in great contrast to pathways when no host-factor is present (Fig. 6A). To demonstrate that this turning behaviour was not directed movement (a taxis) we tested crabs in a trough entirely filled with labelled host-factor, again by introducing host-factor at the same rate from all four drip-tubes and assuming that under these conditions no concentration gradient or interface exists in the trough. As Fig. 6C shows, these pathways show repeated turning in contrast with controls (Fig. 6A) in which no host-factor was in the system.

Several workers have shown that in klinokinetic

responses frequency of turns may be directly proportional to intensity of stimulation (Dethier, 1957). This is true of the responses of *P. chaetopterana* to host-factor[*] "Full-strength" host-factor (4 hosts in 3000 c.c. of labelled sea-water overnight) was diluted with fresh sea-water to make 10 per cent. and 1 per cent. solutions. Twelve crabs were tested with increasing concentration, in standard tests in which host-factor was restricted to the central stream. Pathways were analysed for the number of turns/cm. of track after entrance to host-factor. A turn was defined as any change in direction equal to or greater than 30° from the immediately previous direction, this being the minimum change in direction which could be measured with accuracy from the track of an animal which moves as does this crab. Fig. 7 shows the data in graphic form: C on the abscissa is for the data from the pathways of the 12 animals when tested in sea-water controls. As can be seen, turns/cm. of track increased with the concentration of host-factor.

Chemotaxis

Examination of a positive such as that shown in Fig. 6B indicates that crabs appear to be "behaviourally trapped" within the margins of the critical area.

Analysis of the pathways from our total of 219 tests in which host-factor was restricted to the critical area shows a total of 375 crab-departures from the area, after the crabs had once entered it (Table I). The crabs returned in 215 cases and failed to return in 160. Assuming that crabs after any one departure have an equal chance of moving away from or re-entering the critical area, we may conclude that they re-enter more frequently than they depart, such numbers occurring by chance with a P of <0·01 (as determined by a standard chi-square test).

* Recently, similar behaviour has been observed in *Pinnixa tubicola* Holmes, a commensal of the terebellid *Eupolymnia crescentis* Johnson in Puget Sound.
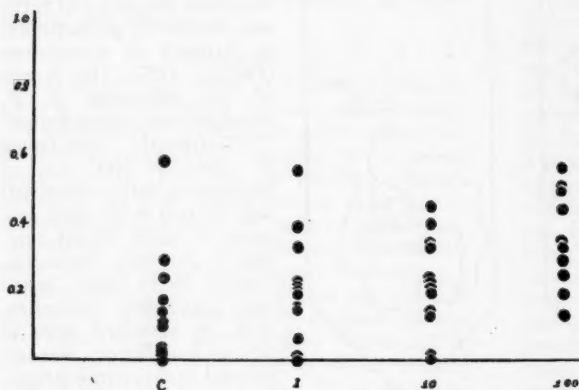
Fig. 7. Turns/cm. of track in pathways of 12 *P. chaetopterana* vs. per cent. concentration of host-factor. C indicates sea-water controls.

One would wish to know whether once crabs have entered host-factor there is a tendency for them to move toward the source. From the above sample of 219 runs in which host-factor was restricted to the central area we eliminated all runs in which crabs (perhaps because of high threshold, refractoriness after several runs or sensory adaptation) showed little response or tendency to be "physiologically trapped." This gave us 179 strong positives which we analysed for net displacement. Of these there was net displacement toward the source in 95, away from the source in 73, neither away from nor toward in 11. There is apparently no significant difference between the number displaced toward the source (95) and the number not so displaced (84). P=0·50.

Rheotaxis

Controls in which no host-factor is present give no evidence that a positive rheotaxis is a

factor in governing behaviour of these animals in the slow currents of the trough (Fig. 6A).

In order to be certain that the tendency of animals to remain in the critical area did not in part depend upon a response to current induced by the presence of host-factor, we analysed the net displacement of animals from 18 pathways described in the trough full of labelled host-factor which is without gradient. In each test a straight line was drawn from the point where the animal was introduced to the point where it first encountered the edge of the trough, at which contact responses affected behaviour. This line represents the sum vector of all the displacements carried out by the animal in reaching the edge. Fig. 8 shows the angles of these vectors from the 18 tracks. There were 9 vectors within 45° of the X-axis and 9 within 45° of the Y-axis. There are 11 vectors pointing in the upstream direction and 7 in the downstream direction.

Specificity of Responses

In Table I we can compare the relative effectiveness of sea-water containing different polychaetes to affect the behaviour of the crabs, by observing the relative tendencies of crabs to be "behaviourally trapped".

It will be seen that crabs collected from *Chaetopterus* are as effectively trapped by the alternate host *Amphitrite* as by *Chaetopterus* (29 returns vs. 9 failures to return—P by standard chi-square test <0·01). Pathways made against *Nereis* and *Arenicola* show no significant response to these worms. In all tests approximately the same total weight of polychaete was used.

Table I.

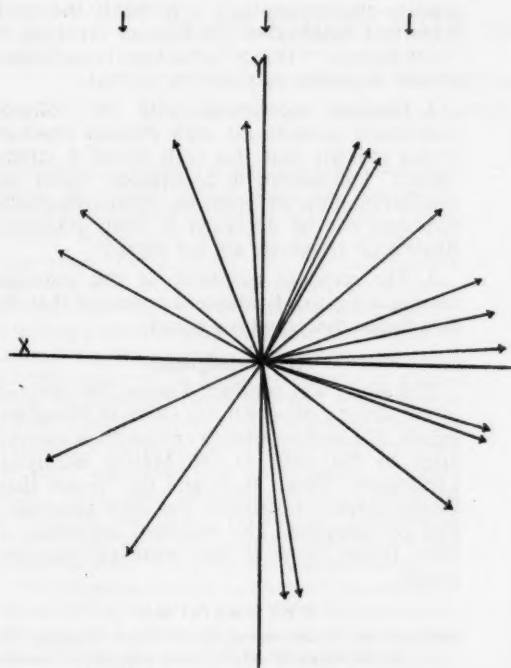| Labelled sea-water from	Total tracks	Crab departures	Returning	Failing to return	P
Chaetopterus	219	375	215	160	<0·01
Amphitrite	19	38	29	9	<0·01
Nereis	28	33	9	24	not sig.
Arenicola	44	46	4	42	not sig.
Sea-water	35	37	3	34	not sig.

Fig. 8. Angles of vectors of net displacement from tracks of 18 crabs in trough filled with fluorescein-labelled host-factor.

Nature of Host-factor

A number of preliminary tests were conducted to give some information concerning the nature of host-factor:

(a) After hosts had been maintained in non-circulating, cooled and aerated sea-water overnight, they were removed. This sea-water was then tested for effect on behaviour. The water was found to elicit typical responses 24 hours after removal of the worms, indicating relative stability of the host-factor at 20°C.

(b) Host-factor-accumulate (4 worms in 3000 c.c. overnight) was heated to 90°-95° for 20 minutes, cooled and tested. All traces of activity had disappeared. Crabs used in these tests gave standard responses in non-heat-treated controls immediately after each test.

(c) Four hosts were placed in a large dialyzing bag and the bag suspended overnight in the test beaker. In the morning water from the beaker (the dialyzate outside the bag) had no effect on

crab behaviour. When the bag was opened and the contents entered the system, the crabs showed standard responses.

(d) Approximately 3000 c.c. of "host-factor-accumulate," from which hosts had been removed in the morning, was divided into two equal parts. One part was treated with trypsin (final solution 0·1 per cent.) for 100 minutes prior to testing. A 0·1 per cent. control solution of trypsin in sea-water was also made up. Four crabs were each tested in four runs (for a total of 16) as follows: (1) in host-factor; (2) in sea-water plus trypsin; (3) in host-factor plus trypsin; (4) in host-factor a second time. In the 16 runs, 1 and 4 were always positive, while 2 and 3 were always negative. Finally, the pH of a 200 c.c. portion of host-factor, previously tested to insure activity, was adjusted to about 7·0. Then $(NH_4)_2SI_4$ was added to make an 80 per cent. saturated solution. The solution was then placed for about 20 hours in a 5°C. cold room. When subsequently examined, only a trace of material, if any, was salted out.

All these tests would indicate that "Chaetopterus-factor" is a relatively time-stable protein.

Discussion

P. chaetopterana shows a marked akinesis when it first encounters host-factor, after which (perhaps with brief sensory adaptation) a "direct" klinokinetic response appears. The intensity of the latter has been shown to be proportional to the concentration of host-factor; with a rise in concentration there is an increase in the rate of random turning.

Analysis of the data of passages through the interface by crabs indicates that at this point of steep gradient they are effectively trapped and return more frequently than they depart. But there does not appear to be a significant tendency for the animals to be displaced toward the source in any of our experiments. We must conclude that if chemotactic responses are of importance in the orientation of the commensals toward their host in shallow gradients, we have been unable to demonstrate the fact. It is entirely possible that such responses occur at high concentrations or in steep gradients, as is indicated by the behaviour of the crabs at the interface of the central stream.

One might suspect that a rheotactic response would be of some importance in an animal which lives commensally with a tubicolous current-

producing host, but such a response does not appear to exist in this crab.

It is reasonable to propose, however, that an effective aggregating mechanism exists in *P. chaetopterana* as a result of its specific "direct" klinokinesis in host-factor. Personal communication with a number of workers who have been interested in such responses (Fraenkel, Wigglesworth, Dethier, etc.) indicates that there has been no experimental demonstration of the efficacy of a "direct" klinokinesis (rate of turning proportional to intensity of stimulation) in bringing about aggregation *at* or *near* the point of highest concentration or intensity of stimulus. In effect, any mechanism which reduces the length of straight runs (the net displacement) in a randomly turning organism moving at constant linear velocity will be as effective a "delaying" mechanism as an "inverse" orthokinesis in which linear velocity decreases with a rise in intensity of stimulation. There is some agreement that aggregation of "directly" klinokinetic animals will occur near the point of highest intensity only if they are slow adapting and that rapidly adapting animals would under such conditions tend to aggregate away from the highest intensity. Clearly, further experimental investigation of these phenomena is in order.

One may, nevertheless, assume that if such an aggregating mechanism existed in a symbiotic animal and if this was reinforced by strong directed responses at steep gradients, the animals would possess a most effective "host-finding" mechanism.

Finally, our experiments have indicated that the specificity of response is probably limited to the hosts of the species concerned, but it is of interest to note for a second time (Davenport, 1953) that a commensal collected from one host species responds strongly to an alternate host not closely related to its own. Preliminary investigations of the nature of the chemical agent concerned in recognition indicates that for the first time we have found an agent which is stable enough for further and more detailed investigations of its nature to be feasible. The evidence is that it is proteinaceous.

Summary

1. A new apparatus is described for the investigation of the responses of aquatic animals to chemical agents.

2. With the apparatus it has been demonstrated in exploratory tests that in the facultative commensal polychaete *Podarke pugettensis* a positive chemotropotaxis is probably the most important behavioural mechanism involved in "host-finding." "Direct" orthokinetic and klinokinetic responses may aid the animal.

3. Detailed experiments with the obligate commensal pinnotherid crab *Pinnixa chaetopterana* indicate that this crab shows a strong "direct" klinokinesis in host-factor. Under the conditions of our experiments, chemotropotactic responses can be discerned in steep gradients. Rheotactic responses are not present.

4. The response specificity of this crab has been investigated. Evidence is presented that the host-factor concerned is a protein.

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COMMENTS ON REDIRECTION WITH EXAMPLES OF AVIAN COPULATIONS WITH SUBSTITUTE OBJECTS

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Animals sometimes direct activities toward an object or animal other than the one which normally releases a particular behaviour. This is termed redirection and has been defined by Moynihan (1955) as follows: "autochthonous activities of a drive directed toward an object or animal other than the one releasing and usually directing them (although the releasing object or animal remains available, or partly available, as a potential goal at the time)." Incidentally, recent work by Andrew (1956) and Van Iersel & Bol (1958) strongly suggests that the term "allochthonous" may no longer be valid, thus rendering the term "autochthonous" unnecessary. Furthermore, until more information is accumulated bearing on the causal factors for phenomena such as redirection and displacement these terms should be used in a purely descriptive sense.

Our experiences with redirection (examples of which follow) have led us to feel that these actually may be best considered as reactions to subnormal stimuli rather than a separate phenomenon.

There are numerous reports of redirected aggression in the ethological literature, but published examples of redirection involving other tendencies are relatively rare. However, there are scattered references in the ornithological literature which seem to be examples of redirected copulations.

A male *Agapornis fischeri* (Fischer's Lovebird) was placed with a female of the same species. The male came into sexual condition earlier than did the female and demonstrated intense precopulatory behaviour which initially achieved little in the way of overt sexual responses from the female. She, in fact, seemed to largely "ignore" the prolonged and intense displays of the male (females of the *Agapornis* species are commonly somewhat aggressive toward sexually behaving males). The male frequently tried to mount the female. She responded to this by being aggressive or by merely sidling out from under him while he still had one foot on the perch. At this point the male would scratch his bill (a common and more or less ritualized act, depending on the species, of *Agapornis*).

After a few days of intermittent activity of this type the male began to culminate his bouts of precopulatory behaviour with copulatory movements on the perch as close to the female as he could get. The movements were normal in every respect and would last from two to four minutes at a time (such prolonged copulations are normal to *Agapornis*). These copulations were made immediately after the male demonstrated intention movements to mount the female and was thwarted because she did not assume the soliciting posture or because she avoided and/or threatened him. Copulation intention movements of varying degrees of completeness were made almost anywhere on the single perch (a piece of tree branch about three-quarters of an inch thick and two feet long). The female, in retreating along the perch from the male's advances, usually ended up against the far side of the cage. About three inches from this end of the perch was a fairly large protuberant knot. It was upon this knot that the male performed the complete copulations while crowding against the "indifferent" female.

The female clearly released and directed the normal precopulatory displays and perhaps even released the copulatory response but she failed, at least, to provide the proper stimuli for copulation itself (the soliciting posture). She also commonly attacked him if he persisted in his attempts to copulate. Hence the male responded to the convenient knot. The female was perhaps utilized only to the extent of providing close contact with the male's side as he copulated.

A male *Hylocichla mustelina* (Wood Thrush) was placed in a large aviary and a female of the same species was in an adjacent aviary. The two birds were thus in full view and hearing of one another and were separated only by the wire netting. The male displayed both visually and vocally to the female. He made repeated attempts to get at the female through the netting. The early attempts to do this were clearly stimulated by his attack tendency but this gradually changed until it appeared that he had become primarily stimulated to behave sexually. Various activities, presumably displacement activities,

occurred as a result of this thwarting of the sexual and aggressive drives in the male. These were primarily bill-wiping and various preening movements. Finally, he began to make frequent copulation intention movements while on the ground and as close to the female as possible. When his tendency to copulate was particularly strong he conducted perfectly normal copulatory motor patterns (except of course he could not lower his tail the usual amount) on a smooth rounded stone about an inch high and about three inches wide by four inches long. This and the more frequent intention movements were performed many times over a period of days.

Again, in this case, the female provided the external stimuli releasing and directing the normal precopulatory displays but since she was not available (intervening wire netting) the male responded to another object (a convenient stone); as did the male *Agapornis* to the knot.

A male *Setophaga ruticilla* (American Redstart) was kept in the same cage with a female, both of which were hand-raised from the age of six days. At the age of about three months he began to sing the advertising song frequently and to direct "courtship" displays to the female. These displays usually culminated in copulation attempts. He often got as far as placing both feet on the female's back, but never brought his tail down far. On several occasions he dismounted without any detectable aggression from the female. The female's attitude was sometimes merely "indifferent" but in other instances she either moved away along the perch or threatened with open gape at the male. After these unsuccessful copulations, he would chase the female around the cage for a few seconds or merely resume feeding. Bill-wiping was observed immediately after two of these attempted copulations.

After three months of sporadic behaviour of this type he was seen to attempt copulation with a pile of dried droppings about one inch high which had accumulated on a perch. Unlike the observations on *Agapornis* this was never observed to follow an unsuccessful copulation attempt with the female, but generally occurred after a period of no overt sexual activity. On one occasion he immediately attempted to copulate with the female after the redirected copulation. He always sang before mounting the pile of droppings and often got as far as placing both feet on it with wings fluttering, pecking it, and then dismounting. He continued these redirected copulations for a month and largely gave up the

copulation attempts with the female, although he still directed "courtship" displays to her.

When copulation was thwarted, the male Redstart performed other activities such as feeding and chasing the female which would seem to indicate a lowering of the tendency to behave sexually or the increase of other tendencies relative to sex. Presumed displacement activities were rarely seen to follow this sexual thwarting. However, after a period of thwarted sexual activity another object in the cage became a source of stimuli directing, or possibly also releasing, copulatory activity and he largely gave up copulatory attempts directed to the female and responded instead to the pile of droppings.

That this male was a young, inexperienced bird probably contributed to the fact that he was able to utilize the substitute object with such facility. It may even have been possible that the pile of droppings gradually, through learning, came to have releasing (as well as directing) properties of its own.

Young (1949) observed a male Robin, *Turdus migratorius*, which tried to copulate with a mound of earth after being threatened by a female that he was approaching. The male then approached the female again and was rejected. He then tried to copulate with a crumpled newspaper. Finally, he approached the female once more and she squatted and copulation took place. The female then attacked and chased him.

The male in this instance was thwarted by the fact that the female was not sexually receptive when he initially approached (in fact she actually threatened him). He then attempted copulation with the nearby substitute objects—the mound of earth and the newspaper before the final copulation with the tardily receptive female.

Simon (1940) found that strutting cock Sage Grouse, *Centrocercus urophasianus*, frequently tread a pile of earth as if they were copulating with it. In fact this is of such frequent occurrence that there is a legend among Wyoming "old timers" that the females of this species are fertilized externally. The females frequently feed in the cocks' strutting ground and it was believed that they ate the semen which the males deposited and thus fertilized themselves !

Discussion

In all three of the cases that we observed the females released at least some of the precopulatory behaviour of the males but were unavailable as copulation partners. In *Agapornis*, *Turdus*, and *Setophaga* the females were not

available because they did not provide stimuli directing copulation to themselves (or actively prevented it through their aggression). In *Hylocichla* the female was unavailable because of the intervening wire netting.

All of the substitute objects used seemed to be non fear-provoking protuberances contrasting with the rest of the immediate surroundings. It seems that although copulation is a comparatively complex act the substitute partners may be very simple with but a few critical properties similar to those characteristic of soliciting females.

The males behaved somewhat differently in approaching the substitute objects than when approaching the females. There were no courtship displays by *Turdus* or *Setophaga* directed toward the substitute object. The *Agapornis* male regularly performed the usual bill scratching displacement activity upon being prevented from performing the act of copulation. Later, when he began performing copulations with the unresisting knot, the displacement activities understandably disappeared in this context. The male Wood Thrush likewise abandoned displacement behaviour after beginning his redirected copulations.

The problem arises as to the difference between reactions to subnormal stimuli and what has been termed redirected activities. In considering the nature of the stimuli which are operating in redirection, one must attempt to look at them from the animal's "point of view". Stimuli which appear subnormal to us (by their very form) may not "appear" this way to the animal. In the above cases of redirected copulations in *Agapornis* and *Setophaga* the females rendered themselves unavailable to the males by their behaviour. It is probable that the females, under the circumstances described, presented stimuli less optimal for promoting copulation than did the substitute stimuli which were finally utilized. The best method for determining whether one set of stimuli is less optimal than another is to give the animal a choice between them. The redirected copulations described are actually natural experiments of this type. The results would suggest that an unreceptive or actually aggressive female may be, under certain conditions, a less optimal object than is a stone, knot, or a pile of droppings.

The only apparent distinction between redirection and reactions to subnormal stimuli is the fact that in redirection the usual stimulus situation initiates the behaviour (often appetitive behaviour). A situation might arise, for instance, where an animal stimulates another to react, but then becomes unavailable (e.g., leaves) as an adequate stimulus for the continuation of the usual course of events, and the first animal then continues to react to some substitute object. This, however, would not be redirection according to Moynihan's definition ". . . . (although the releasing object or animal remains available, or partly available, as a potential goal at the time)". It seems to us, however, that the difference between what has been termed redirection and reactions to suboptimal stimuli is virtually non-existent. Therefore, we suggest that re-direction be considered behaviour in which an act of overt behaviour is initiated by the normal stimulus (i) or stimulus situation and is then directed to a substitute object or animal upon the subsequent unavailability of the original source of stimulation.

An animal normally experiences little difficulty in finding a suitable object to which aggression may be redirected. In birds, for instance, pecking is an almost universal aggressive act and any environment will usually provide substitute objects for pecking (other animals, the substrate, vegetation, etc.). This is probably one of the reasons why redirected aggression is so much more common than are other types. If redirection is to occur in an animal with a thwarted sex drive, the animal must find an object close by which will serve as a substitute sexual partner. Such an object is necessarily more complex and other individuals are not likely to co-operate. The relationships among reactions to subnormal stimuli, redirection, and displacement are not clear. Apparently, if the animal cannot perform an activitated behaviour, either to the normal situation or to an available subnormal situation (redirection) the next most available (not necessarily the next strongest) drive will be disinhibited (see Van Iersel & Bol, 1958) and displacement will result.

Summary

1. Redirected copulation is described in the Fischer's Lovebird, *Agapornis fischei*; Wood Thrush, *Hylocichla mustelina;* and the American Redstart, *Setophaga ruticilla*.

2. Redirected copulation occurs when the male is thwarted sexually because the female (providing the initial stimulation) becomes unavailable for some reason during the normal course of a precopulatory sequence.

3. The substitute object resembles a soliciting female in that both are non fear-provoking protuberances in the male's immediate surroundings.

4. In one case, that of the Redstart, the substitute object apparently came, through learning, to have releasing (as well as directing stimuli) of its own.

5. Apparently redirection rather than displacement may be performed if the immediate surroundings provide an adequate substitute for the suddenly unavailable usual object or situation.

6. Redirection is considered to be a special type of reaction to "subnormal" stimuli, although the activity is initiated by "normal" stimuli.

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SOCIAL INHIBITION OF THE DOMESTIC CHICK'S RESPONSE TO VISUAL FLICKER

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It has been reported elsewhere (James, 1959) that newly hatched domestic chicks will approach and stay by a flickering light, and that they will follow objects which have previously been associated with such a light source. It has also been shown (James, 1960) that the attractiveness of flicker for the domestic chick diminishes with age at first exposure, in much the same way as does the attractiveness of a moving object in conventional imprinting experiments (Jaynes, 1957).

Another way in which this analogy between imprinting and the response to visual flicker may be examined further is suggested by some recent results of Guiton (1959), who has shown that communally raised chicks are less likely to imprint on an artificial lure than are chicks reared in isolation. If the process underlying the response to flicker is similar to that of imprinting, chicks which have been reared together should be less responsive to flicker than chicks raised in isolation. The experiment described below was made to test this hypothesis.

Subjects

Sixteen Barred Rock chicks were obtained from a commercial hatchery when they were approximately 48 hours old. On arrival, 10 of these chicks, chosen at random, were housed in pairs in opaque fibreglass mouse cages, and the remaining 6 chicks were raised in isolation in identical cages. Food and water were continuously available and the cages were heated by means of electric lamps placed under the sheet of hardboard on which they rested.

Apparatus

The runway in which the chicks were trained and tested has already been described (James, 1959). It consisted essentially of a 10-ft. long hardboard box, in each end of which five ¾-inch holes were drilled in a diamond pattern (like the "five" on a tilted die). The diagonal distance between the holes was 3 inches, and the centre of the diamond was 6 inches from the floor of the runway. A stroboscopic lamp, giving 10 flashes per second, was placed behind

one or the other of these sets of holes. A blue polythene ball was fixed to the wall in the centre of the holes through which the light flashes could be seen. No light was visible through the holes at the other end of the runway. One of Gellerman's trial orders (Stevens, 1951) was used to determine the end of the runway at which the light should flash on any given trial.

Procedure

All the chicks were run individually, and given two 5-minute trials a day for 5 days, starting on the day of arrival. On each of these trials the chick was placed in the centre of the runway and its distance from the end of the runway at which the light was flashing was measured to the nearest 3 inches every 30 seconds. The mean of these 10 distances was taken as the chick's score for a given trial.

Results

The median scores of the two groups of chicks on each trial are given in Fig. 1. The difference between the groups on the last day of training (trials 9 and 10 combined) is significant at the 0·025 level (Mann-Whitney test; $U=11$). The same result was obtained when the experiment was repeated under slightly different conditions (60-watt lamp flashing at 160 flashes per minute; holes at opposite end of the runway illuminated by steady 60-watt lamp; social group $N=8$; isolated group $N=6$; Mann-Whitney $U=8$, $p=p·021$).

Summary and Conclusion

This result indicates that domestic chicks which have been given an opportunity to imprint upon one another are less attracted to a flickering light than chicks which have been raised in isolation from the age of about 2 days. It thus lends further support to the hypothesis that imprinting and the response to visual flicker depend upon the same mechanisms.

As is commonly the case in this situation, the isolated chicks were relatively quiet in the runway. The socially reared chicks emitted loud and continuous distress cries, in the way in which
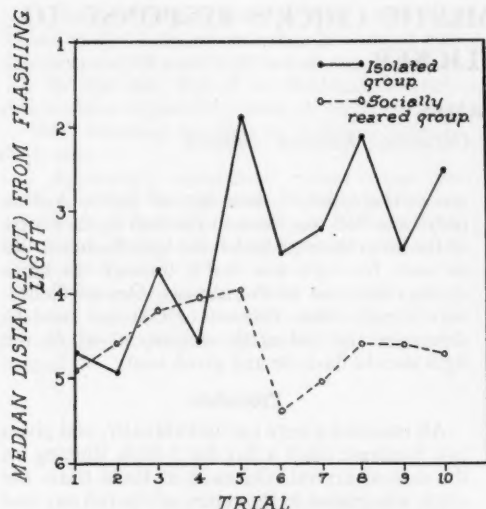
Fig. 1. Median distance of chicks in the isolated and socially reared groups from that end of the runway at which the light was flashing on and off, as a function of trial number.

they sometimes (but not always) do when an attempt is made to extinguish the response to flicker (Abercrombie & James, 1960). There was, however, no evidence that the socially reared chicks actively avoided the end of the runway at which the light was flickering.

One other aspect of the results deserves brief notice. The level of responsiveness of the isolated chicks is lower than that observed in other experiments (e.g. James, 1959) which have been conducted under similar conditions. The scores of the chicks in the replication experiment were virtually identical with those shown in Fig. 1, and so it seems unlikely that this effect is simply due to sampling error. Since, however, there is no indication in the notes kept during these experiments that any unusual circumstances were prevailing at the time, an alternative reason for this effect cannot be suggested at present.

Acknowledgment

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USE OF FISH SCHOOL FOR CONDITIONED RESPONSE EXPERIMENTS

By CHARLES P. O'CONNELL

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A conditioned response experiment was carried out in January 1956 on the Pacific sardine, *Sardinops caerulea*, to assess the feasibility of applying such a technique to this and other clupeoid fishes in the study of their sensory capacities. The object was to determine (1) whether a school of sardines could be conditioned as a unit, and (2) whether the conditioned response of the school would be adversely affected by the replacement of mortalities with untrained specimens.

The experiment was conducted, as a part of the California Co-operative Oceanic Fisheries Investigations, by the Bureau of Commercial Fisheries Biological Laboratory at La Jolla. The staff of the public aquarium at Scripps Institution of Oceanography was most co-operative in providing space and facilities.

Methods

Twenty-one adult sardines, taken from a display aquarium containing three to four hundred sardines, were placed in a hexagonal concrete tank ($46 \times 27 \times 49 \times 15 \times 49 \times 27$ inches and 30 inches deep) of 453 gallons capacity, which was supplied with a continuous flow of filtered sea water from an open pump and reservoir system. These fish had been established for some time in the display aquarium on high-protein food pellets, as recommended by the results of Farris (1956). The fish would swim rapidly through these pellets and pick them off individually as they dropped slowly through the water. No attempt was made to pick up any pellets that reached bottom.

To condition the experimental school, a five-foot length of board with a feeding port near the centre and a light suspended to one side of the feeding port was placed across a wide-angled corner of the aquarium. Another five-foot length of board, nailed at right angles to this baseboard, formed a vertical shield to hide all but the upper portion of the observer's face from the view of the fish. Violent movements were avoided, and at no time did the fish alter their behaviour when an observer appeared behind the shield. The light source, a six-inch tubular, 25-watt frosted bulb mounted so that the lower

end projected below the surface of the water, was operated by a mercury switch located on the upper surface of the baseboard. Ambient illumination during the day was indirect sunlight from the windows of the building, none of which was immediately over the aquarium.

The school was subjected to twenty-one periods of conditioning over a space of twenty-two days, after which the aquarium was relinquished for another purpose. There were usually two periods of conditioning a day during the first half of the experiment and only one a day during the last half. The greatest interval between conditioning periods was 69 hours, from the morning of the thirteenth to the morning of the sixteenth. This was unavoidable, but fortunately it did not retard the progress of the experiment.

Each conditioning period consisted of ten trials carried out as follows: The light was turned on for five seconds. As it was switched off, about thirty premoistened food pellets were dropped through the feeding port. This food reached the bottom in six or seven seconds if not eaten. The light remained off for two minutes between trials, but as the experiment progressed it was sometimes necessary to lengthen the "light off" period to allow the feeding frenzy to subside. The light was switched on only when the fish were moving along the far side of the aquarium.

Dead or near-dead fish were removed from the aquarium after the conditioning period when they were discovered. No more than two such removals were ever replaced at one time with healthy specimens from the larger display aquarium. The presence of dead or near-dead fish in the aquarium during a conditioning period had no noticeable effect on the reactions of the other fish to the light and food.

After each trial the reactions of the fish to the light and to the food were recorded in some detail, and after the experiment was terminated, each of these entries was rated according to the following scale:

0. *No reaction to the light. No reaction to the food.* Sometimes the fish started slightly when the light went on or off.

Table I. Rated Response of Sardine School to Conditioned Stimulus. Each Conditioning Period Includes Ten Trials and is designated by a Date-Time Group. The rating scale is defined in the text. The letter notations indicate the following: *a* Outside disturbance, *b* Substandard food ration, *c* Food dropped during "light on", *d* Failure to record.

Periods (Date-Time)	Trials										Mean
	1	2	3	4	5	6	7	8	9	10	
041600	0	0	0	0	0	0	0	0	0	0	0
050930	0	0	1	1	0	0	1	0	1	1	·5
051600	0	1	1	2	2	2	2	2	2	2	1·6
060930	2	2	3	2	2	3	3	3	a	a	2·5
061615	2	2	3	3	2	2	3	3	0	3	2·3
071615	2	2	3	3	3	3	2	3	2	2	2·5
081615	3	4	4	4	3	3	2	3	3	4	3·3
090930	b	b	b	b	b	2	2	3	3	2	2·4
091615	1	2	2	3	2	2	3	2	4	4	2·5
100930	3	3	3	3	3	4	4	3	3	2	3·1
111100	2	4	4	4	4	4	4	4	4	4	3·8
121100	1	3	3	4	2	2	4	4	4	a	3·0
131145	1	2	3	3	4	3	4	3	2	c	2·8
160845	3	4	4	4	3	d	4	4	5	5	4·0
170945	3	5	5	5	6	6	6	6	6	6	5·4
181130	2	5	5	5	6	6	5	5	5	5	4·9
191130	3	4	5	5	5	5	6	5	6	6	5·0
201330	4	6	6	6	6	6	5	6	5	5	5·5
211500	2	5	5	5	5	6	6	5	6	5	5·0
230930	4	5	6	6	6	5	6	5	6	6	5·5
241100	2	5	5	6	5	6	6	5	6	6	5·2
251145	2	5	5	4	5	5	6	6	6	6	5·0

1. *No reaction to the light. Weak reaction to the food.* Less than one quarter of the fish noticed the food. Very few pellets were taken.

2. *No reaction to the light. Moderate reaction to the food.* Excitement was generated as soon as any of the fish detected the food, but many pellets were missed.

3. *Slight excitement during "light on" period. Strong reaction to the food.* Most of the food was taken.

4. *Moderate excitement during "light on" period. Instant reaction to the food.* There was a definite increase in the rate of swimming while the light was on. Virtually all of the food was taken.

5. *Strong allelomimetic* reaction during "light on" period. Instant reaction to the food.* The fish doubled or tripled speed and tightened up the school while the light was on. Virtually all of the food was taken.

6. *Strong allelomimetic turn towards the feeding port during "light on" period. Instant reaction to the food.* Sometimes the school made as many as three high speed circuits around the

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*Allelomimetry is a term used by Scott (1958) to denote "... behaviour in which two or more animals *do the same thing*, with some degree of mutual stimulation."

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tank before the light was turned off. Occasionally there was evidence of confusion as they passed under the feeding port without receiving food. When the light went off they often shortened their circle to reach the feeding port. Virtually all of the food was taken.

Results

The response ratings for each trial are shown in Table I. The trials marked with letters were not given ratings because of outside disturbance or irregularities in the procedure, as described in the table heading. Fig. 1, in which the mean rating and first trial rating for each conditioning period are plotted against time, clearly illustrates the improvement in the conditioned response as the experiment progressed. It should be noted, however, that some degree of relearning was involved in every conditioning period. Response in the first trial was always lower than the average for the entire conditioning period, even though they both increase with time. In the earlier conditioning periods the response was not much better in the second and subsequent trials than in the first, but in the later periods response was almost invariably strong from the second trial on.

By the end of the experiment, 12 fish, or 41 per cent. of the 29 used, had died (Table II). The original school was composed of 21 fish, and
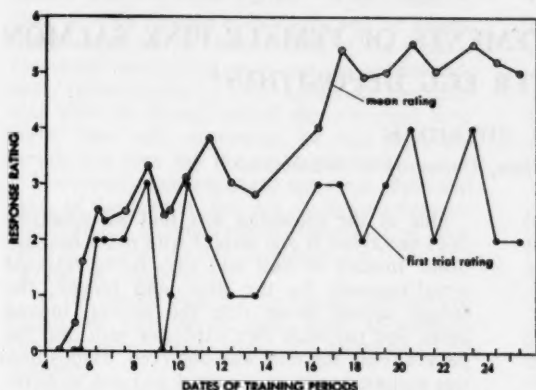
Fig. 1. Curves of mean rating and first trial rating during the course of the experiment. The interval between dates represents twenty-four hours. Points are plotted at the training period hour. See text for a n explanation of the response rating scale.

Table II. A Record of Mortalities and Replacements during the Conditioning Experiment.

Date	7	11	16	19	20	24	Total
Mortalities	2	1	1	4	1	3	12
Replacements	2	1	0	2	1	2	8
Population	21	21	20	18	18	17	17

there were twelve deaths, eight of which were replaced as they occurred. Failure to replace the mortality of the 16th was an oversight. Of the four removed on the 19th, two were dead and two moribund. Of the three removed on the 24th, two were dead and one moribund. Those that were removed after the 18th exhibited the symptoms attributed by Parrish, Blaxter & Holliday (1958) to epidermal damage and consequent disturbance of osmotic balance in herring.

Additions to the school during the course of the experiment, as given in Table II, did not have an noticeable effect on school response, as given in Table I and Fig. 1. As a matter of fact, each specimen added to the school was observed to act entirely in unison with the school from its first trial.

This experiment demonstrates that the conditioned response technique can be applied successfully to a small school of sardines, and that a moderate replacement of mortalities will not alter the conditioned response of the school. Since mortality in aquariums is a considerable problem with the Pacific sardine, the probability of carrying an experiment to fruitful completion would be much greater with a trained school than with one or a few trained specimens.

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FREQUENCY OF DIGGING MOVEMENTS OF FEMALE PINK SALMON BEFORE AND AFTER EGG DEPOSITION*

BY W. L. SHERIDAN

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The pink salmon (*Oncorhynchus gorbusha*) has a life cycle of two years. This species spawns in great abundance in streams draining into the North Pacific Ocean and Behring Sea where it is of considerable economic importance.

Although good descriptions of the spawning of other salmonids have been published, other than Wickett (1959) we do not know of any account of the spawning act of the pink salmon. Two spawning acts, observed by the writer,† are thought worth reporting for two reasons: because of lack of description in the literature and chiefly because of the marked change in the frequency of digging movements of the female before and after oviposition.

Comprehensive accounts of the spawning act of some species of salmon have appeared from time to time. Jones & King (1949) summarized literature pertaining to the spawning of the Atlantic salmon and Briggs (1953) did the same for the Pacific salmon. According to these general descriptions, the female salmon first chooses a location (sometimes after trial digs) and digs a pocket (or nest) in the gravel. To make this pocket the female turns on her side and executes a series of swimming movements. The vigorous downstroke of the posterior half of the body thrusts the water against the gravel with sufficient force to loosen it and the upward flexion further assists the movement downstream of the displaced gravel by upward suction (Jones & King, 1949). After the pocket has been prepared, the male and female settle into it. With mouths agape eggs and milt are released simultaneously. After egg deposition and fertilization, by dislodging gravel around the sides and upstream part of the pocket, the female covers the eggs and starts another pocket, generally upstream from the first. The eggs are not disturbed during the cover up period because they adhere to stones in the bottom of the pocket.

One of the spawning acts that we observed does not agree in one respect with other descriptions. Instead of milt and eggs being released simultaneously by the male and female, the female settled down into the pocket, moved aside, and the male then expressed milt into the pocket (only the milt was observed, oviposition was assumed to have occurred and ova were recovered later). It is possible that the recovered eggs had been deposited at a previous simultaneous spawning by a male and female. This is doubtful however, for two reasons. First, the increase in frequency of digging movements immediately following apparent oviposition is very strong evidence that oviposition occurred. Second, eggs were recovered from the exact place where the female had been digging. No females had been observed digging in this area before and it is extremely unlikely that the same female would dig a second pocket over the first which she had just covered. Separate, rather than simultaneous, spawning is certainly not a normal occurrence with other species of Salmonids. It is doubtful if it is normal with the pink salmon because in the second observed spawning act two males, one on each side of the female, both expressed milt.

The first spawning act was observed by Dr. E. K. Bjornerud and the writer in Cabin Creek, a small stream in Southeast Alaska in 1949. At the time the stream was low (8 to 10 inches deep at the redd site) and clear. Water velocity was 0·8 feet per second.

It was not known how long the female had been digging when observations were started at 1230 hours. From that time, the salmon were observed for 73·1 minutes and certain activities were timed with a stopwatch. For the first ten minutes, the female made digging movements at regular intervals. After 10·3 minutes, the female for a brief moment settled down on the pocket she had made, then withdrew to the left side of the depression which appeared to be about 4 to 6 inches deep. The male swam rapidly in from two feet downstream, settled on the pocket and extruded a milky cloud of milt which hung over and in the pocket, only the topmost layer being

carried away by the current. Approximately three seconds elapsed before milt was no longer visible clouding the deepest part of the pocket. The male then drifted away and did not immediately return (prior to milt expression, the male was with the female all of the observed time while after milt expression he was with the female less than half the observed time).

The female then dug at the upstream edges and sides of the pocket, but not always alternately. For example, during a specific time interval there were 31 digs on the left, 25 digs on the right, and 14 digs at mid-centre.

As the female dug, the depression was seen to slowly fill with gravel until, after 45 minutes from assumed oviposition, no depression was apparent.

Observation was discontinued at 1343 hours because of poor light. The female on capture contained 650 ova while 200 ova, mainly concentrated in one compact body, were recovered from the pocket.

By timing the interval between digging movements, changes in frequency were recorded. The accompanying figure shows the changes in frequency of digging movements of the female pink salmon under observation compared with those of another female when no evidence of oviposition or milt extrusion was observed. Of three

additional females timed for varying intervals prior to oviposition, the rate of digging movements averaged one per minute.

In summary, for this female observed in the spawning act, an average of 78 seconds elapsed between digging movements until the time of assumed oviposition. From oviposition to approximately 10 minutes after oviposition, the average time between digging movements decreased to nine seconds and for the remaining 50 minutes again increased to 39·6 seconds.

A second spawning act was observed by the writer in Indian Creek in southeast Alaska in the fall of 1959. The second observation, with the exception of apparent simultaneous oviposition and milt expression, served to corroborate behaviour observed during the first spawning act.

One female and two male salmon were observed from 1650 hours to 1750 hours. Water depth was 6 to 9 inches at the redd site and water velocity was 1·0 to 1·5 feet per second. Again it was not known how long the female had been digging, but at the beginning of observation the pocket appeared to be 5 to 8 inches deep. One to three males were with the female while she prepared the pocket. At 1738 hours the female settled down into the pocket with a male on either side. Two separate but almost simultaneous clouds of milt appeared briefly.

Digging frequency of this female was less than one dig per minute from 1150 to 1538 hours. Immediately after oviposition the number of digs per minute rose to 9 as the female went through the typical covering routine. For the first twelve minutes following oviposition the female averaged 5 digs per minute.

Discussion

Based on two detailed observations of the spawning act of pink salmon, one in Cabin Creek, and one in Indian Creek, there was an evident increase in frequency of digging movements exhibited by the pink salmon female after oviposition. This changing frequency has been mentioned by a few authors, but seems to have gone unnoticed by most.

Belding (1934) wrote of the Atlantic salmon, "The frenzied digging which follows the extrusion and fertilization of the eggs, is quite different from the leisurely digging in preparing the redd. . . ." Briggs (1953) reported of the silver salmon that the interval between digging
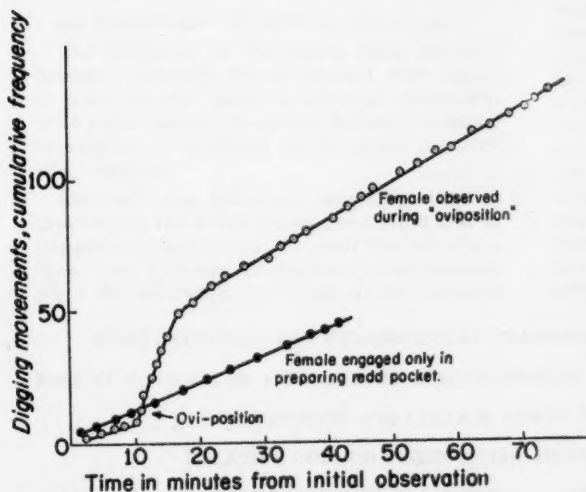
Change in frequency of digging movements of a pink salmon female after oviposition compared with regularly spaced digging movements of another female engaged only in preparing a redd pocket.

movements prior to oviposition was once every two or three minutes and that as soon as the sex products were liberated the female dug with more than usual rapidity. Mathisen (1956) stated that the frequency of digging movements of sockeye salmon females was about one per minute before spawning and increased to from five to ten per minute shortly after oviposition. He suggests that 'this increased digging is an infallible criterion of recently completed egg deposition'.

Two reasons are suggested for the increase in frequency of digging movements after oviposition. First, as long as eggs lay uncovered in the nest, they are subject to the raids of predators, for instance the Dolly Varden trout (*Salvelinus malma*). Second, salmon eggs retain their adhesive characteristics for 20 minutes or less around 55°F.* Attempts made to cover eggs

*Personal communication with Dr. Lauren R. Donaldson of the College of Fisheries, University of Washington.

in a nest after adhesive qualities are lost have resulted in eggs being pushed out by the turbulance created by descending gravel particles.

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PROCEEDINGS OF THE ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR

A meeting of the Association was held in the Institute of Experimental Psychology, Oxford, by kind permission of Professor R. C. Oldfield, on March 30th, 31st and April 1st, 1960. The papers presented on March 30th were devoted to Perceptual Mechanisms in Animal Behaviour, and were presented to a joint meeting of the Experimental Psychology Society and the Association.

Wednesday, March 30th

VISUAL PERCEPTION AND ORIENTATION IN THE DESERT LOCUST. By G. K. WALLACE, *Reading*

This paper raised the problem of visually controlled locomotion in insects. Reference was made to the writings of Fraenkel and Gunn on this topic and it was suggested that their explanations were perhaps particularised in that they applied to orientation to light sources and not to objects. Previous experiments had shown that for locusts stimulation of the retinal units by moving contours in the visual field plays an important part in their behaviour and that this information is collected by a scanning mechanism.

The paper divided orientated locomotion into two main phases:

(1) choice of direction.

(2) maintenance of direction once chosen.

The behaviour of the insects while choosing between different objects showed that choice is made on the basis of stimulus inequality. With equal stimuli, choice may be due to a chance movement of the insect which brings it nearer one stimulus.

Evidence was advanced suggesting that in cases where the insect shows orientation first to one stimulus and then to the other this is due to a lower level response (orientation) being released when the combination of internal and external factors is not high enough to release a higher level response (jumping).

With regard to maintenance of direction, experiments provided no evidence for a centrifugal blocking of irrelevant inputs once a choice is made. The visual information picked up by scanning is different from that picked up when walking and there is evidence that the latter produces deviations from the track initially chosen. Insects approaching a broad object show zig-zag tracks, orientating successively to the two vertical edges of the object. This is thought to be due to distraction by the edge which lies to the side of the insect.

Some preliminary tests with brain lesions were reported. These indicated that the mushroom body calyces in the protocerebrum have an inhibiting effect on activity but no direct effect on orientation. Unilateral lesions of these calyces do not produce circling. Unilateral lesions in other parts of the protocerebrum produce circling to the intact side. Lesions just dorsal to the antennal lobes facilitate the wheeling response, an orientation response to proximate objects placed laterally. Insects with this lesion show the response to objects at greater distances than normal. Pathways in this region may be concerned with the passage of information concerned in the visual control of orientation.

SOME EXPERIMENTS ON INTEROCULAR TRANSFER IN OCTOPUS. By W. R. MUNTZ, *Oxford*.

RECENT FINDINGS ON VISUAL SHAPE DISCRIMINATION IN OCTOPUS. By N. S. SUTHERLAND, *Oxford*.

EYE MOVEMENTS AND COLOUR VISION IN MAN. By D. FENDER, *Reading*.

RELATIVE COLOUR VISION IN THE HONEYBEE. By H. KALMUS, *London*.

The recent revival and expansion by Gehrkre (1948), Land (1959) and others of Goethean notions concerning man's colour sensations in complex situations, made it desirable to look for comparable faculties in another organism. Detailed work by Daumer (1956) on the colour system—in "aperature vision"—of the honey bee suggested this insect as a suitable object.

He showed that the spectrum of light visible to *Apis mellifica* extends further into the shorter range than that of man, but is cut off between orange and red. Like man bees are trichromatic and their colour mixing follows additive rules; but their primaries—yellow, blue and near ultra violet—are different from ours and they also have different complementary colour pairs.

The experiments described here were performed by employing dual illuminations. Bees were trained to a feeder placed on a circular translucent disc, covered on both faces with polygonal patterns including six equally spaced stars in a circle. Different amounts of grey were combined on the upper and lower surface in every pattern element; in particular three stars were painted light grey on both sides, whereas the three alternate stars were black below and white on top. Training was mostly done by covering one of the grey stars with a star cut from coloured (for instance blue) paper, and putting a syrup-filled feeder on top of it. The disc was mounted on top of a lamp case on a vertical axis turning once in a minute. During training

time 6-10 individually marked bees were trained and counter trained with water containing feeders on two of the grey stars, while the other three stars were covered. Training proceeded in fairly dim daylight inside a hut where the whole arrangement was set up. After several hours a trial run usually showed that the bees had learned the colour. Feeding was then resumed for 10 minutes when the (blue) paper star and the syrup feeder were removed. The exposed grey star was then covered and one of the white stars bared and a water feeder put on top. One of the lamps in the case (sodium lamp, incandescent lamp, mercury lamp, black and U/V lamp) or a combination were then switched on. In the resulting dual illumination the whole pattern appeared vividly multicoloured and with sodium lamps the black/white star looked a brilliant blue. In this instance the bees also were "deceived" and hurled themselves at the water feeder. In accordance with Daumer's findings bees accepted only a few of the human matches and their acceptances mostly differed from the observer's. Quantitative data will be published later.

FILM made by G. J. BROEKHUYSEN, *Cape Town,* **ON THE STIMULI ELICITING EGG-SHELL REMOVAL IN THE BLACK-HEADED GULL.** Introduced by N. TINBERGEN, *Oxford.*

Thursday, March 31st

COURTSHIP BEHAVIOUR IN THE *Drosophila obscura* **GROUP.** By R. G. B. BROWN, *Oxford.*

AN EXPERIMENTAL STUDY OF SEXUAL ISOLATION WITHIN THE SPECIES *Drosophila melanogaster.* By S. PEARCE, *Oxford.*

The experiment described by Knight, Robertson & Waddington, (*Evol.* **9**, 1956), was repeated, in order to identify any changes in mating behaviour patterns, produced as a result of selection.

Experimental procedure was modified to avoid:—(1) selections for spatial isolation within the mating chamber, (2) larval competition and possible selection for differential larval survival and early emergence of the selected flies.

Equal numbers of ebony and vestigial flies were used in each generation (110♂♂ and 110♀♀). Selection was in favour of homogamic matings, (vg female × vg male; e female × e male), and against heterogamic matings, (vg female × e male; e female × vg male). A control line in which there was no selection for sexual isolation was maintained and used for comparison with the selected line.

Selection was continued for 40 generations. Quantitative analysis of male and female mating

behaviour patterns of the unselected and selected flies, supports the observed results:—

(1) The degree of homogamic mating in generation 1 is greater than expected from random mating. This is due to the effect of the marker genes, ebony and vestigial on behaviour. Neither mutant male mates as quickly as the wild-type male, and of the two, vestigial is the least successful. This decrease in mating success may be attributed to differences in male courtship behaviour, in vestigial to the absence of the wing vibration component, and in ebony to breaks early in courtship. Reduction in percentage licking in both mutants, and reduction in percentage vibration in ebony, also contribute, but to a lesser degree. Vestigial males are slightly more successful in the mating chamber than expected from studies of single pair matings, due to inclusion with winged forms: the stimuli received by a female being cumulative in effect. Crowding with their own kind does not raise the

activity of either form. Mutant females are slightly less receptive than wild-type females, and vestigial females are slightly more receptive than ebony females. This is mainly because wing-flicking, an important repelling action, is absent in vestigial, but also because lack of wings permits the males to move closer, so facilitating licking and mounting.

(2) Selection has had two effects:—

(*a*) Increase in activity of both mutant males so that copulations occur earlier. This is mainly due to increased percentage licking in the courtship of both mutant males and to increased persistence in the ebony male.

(*b*) Increase in homogamic matings and decrease in heterogamic matings in the selected line but not in the control line. This increase in sexual isolation in the selected line is due to a change in female mating behaviour. Thus selected females repel the opposite males more than do unselected females. Strongly repelling

actions used by both females are kicking with the mid and hind limbs, and twisting of the abdomen. The ebony female, in addition, uses wing-flicking. It is suggested that the differences in ebony and vestigial male courtship behaviour, enables the females to discriminate and to make more repelling movements towards the "wrong" male. The selected males begin to court both females as readily as unselected males, suggesting that they do not discriminate. Selected males do however respond to repelling movements, by breaking off courtship, more quickly than formerly, with the result that they only persist in courting their own females.

It is concluded that sexual isolation at any point depends on the relative success of the males and on the mating preferences of the females. Total selection against hybrids over 40 generations has resulted in changes in mating behaviour patterns. These changes are responsible for the increase in sexual isolation measured during the course of the experiment.

BODY SIZE AND BEHAVIOUR IN *Drosophila melanogaster*. By A. W. EWING, *Edinburgh*.

FACTOR ANALYSIS OF INSECT BEHAVIOUR. By R. R. SOKAL, *Kansas and London*, & H. V. DALY, *Louisiana*.

This study aims at demonstrating the value of multiple factor analysis in a dynamic biological model. Nineteen biological variables were taken from six species of insects. The variables were of two kinds: those involving pulsation of the heart or gut and those involving fleeing reactions when exposed on a glass surface. Six physical variables were measured concurrently with the biological variables. Fifty-five replicate readings were taken. Correlation coefficients were computed among the 25 biological and physical variables. These correlation coefficients were subjected to a complete centroid factor extraction and the results rotated to simple structure by the mass modification of Thurstone's analytical method.

Six factors appeared to cause most of the cor-

relations observed in the study. Only one physical factor affects biological variables, namely, factor (I), the solar energy factor. Another physical factor (VI), which turned out to be a time series factor did not affect any of the biological variables. The remaining four factors were primarily concerned with the flour moth (II) flour moth locomotion (III), the wasp (IV) and the louse (V). The effect of these factors on the 19 variables is discussed. The authors conclude that factor analysis is useful as a method of simplifying a correlation matrix into descriptive components called factors and that in suitable material at least some of these factors could be reified to meaningful physical and biological variables, while the unidentified factors serve to indicate the need for further enquiry.

THE QUANTITATIVE CONTROL OF RHYTHMIC SETTLING MOVEMENTS IN HEMILEUCID MOTHS. By A. D. BLEST, *London*.

THE IMPORTANCE OF MUTUAL STIMULATION IN GROUP FORMATION BY DESERT LOCUST HOPPERS. By P. ELLIS, *Oxford*.

DO DESERT LOCUST HOPPERS RECOGNISE EACH OTHER? By A. PEARCE, *Oxford*.

THE BEHAVIOUR OF THE ATTENDANTS ON THE HONEYBEE QUEEN. By M. D. ALLEN, *Aberdeen*.

Friday, April 1st

SOME ASPECTS OF SOCIAL COMMUNICATION IN BLACK-HEADED GULLS. By G. MANLEY, *Oxford.*

In order to illustrate the communicative capacity and organisation of the display repertoire of the Black-headed Gull, certain displays may be selected for comparison in terms of their information-level and the functional systems they serve.

Some patterns are simple or unitary insofar as each serves a single functional system, carries a single message and is the outcome of a restricted motivational background. Thus ambiguity is impossible with signals such as the Alarm Call and Food-finding Call; the former is given whenever danger threatens when it may be seen to alert others, the latter only in the presence of food when it probably functions as an attractant.

In a number of situations, however, the same (or superficially the same) displays operate in entirely different functional systems, convey dissimilar messages and are the products of dissimilar underlying causal states. The question then arises as to how this can be achieved without social confusion. Displays such as the Oblique, Forward and Upright, whilst clearly valid threat patterns, occur quite as regularly in the sexual context of pair-formation; in the one we have intimidation leading to spacing out of individuals, in the other the opposite effect of distance-reduction and pairing. Close examination of these agonistic responses and the pair-formation Greeting Ceremony (comprising the same displays) reveals that whereas in the former the patterns vary in certain of their components, are used largely independently of each other and without rigid orientation, in the latter each of the three displays has a constant form, is linked in a fixed sequence with the others and performed with parallel orientation. The signal value of the whole sequence is quite distinct from that of its separate constituents.

In the example just considered the displays have been modified in various ways and the similarity is only superficial. In Choking an identical display is used in three functional systems (agonistic, sexual and incubation) when its causation also differs from case to case. It becomes evident from this that the full information conveyed cannot lie solely within the pattern itself, that it is necessary to postulate in the recipient the capacity to distinguish qualifying clauses in the different contexts. Such a capacity I have termed "context-interpretation" and in Choking it is possible to recognize a basic message which in each situation is additionally qualified.

Considering the complete display repertoire of the Black-headed Gull it is apparent that the processes described (modification by form-constancy, linkage, etc.; context-interpretation) have been fully exploited, so much so that displays of a simple, unitary character make up only a small minority.

'NERVOUS ENERGY' IN HYBRID MICE. By S. A. BARNETT, *Glasgow.*

Crosses have been made between mice of four highly inbred strains. The F_1 mice so produced tend to be more active than members of the parent strains; the same applies to an outbred stock obtained by mixing all four strains. The resting metabolic rate (oxygen consumption) is however the same in the inbred and the hybrid mice.

The greater activity is evident more in responsiveness to particular objects than in general movements about the cage. For example, the hybrid mice, unlike the inbred ones, usually gnaw and waste large amounts of the cubed diet on which they are fed. The difference has however been most clearly shown when the mice were presented with paper strips hanging over the side of the cage. The strips are pulled down and made into a nest much more quickly by hybrid than by inbred mice.

Although the strips are made into a nest, the behaviour is not related in a simple way to heat conservation or comfort. The main experiments were done in a room kept at 21°C. When they were repeated in a room kept at −3°C. the hybrids were no quicker than the inbreds to pull down the strips. Thus in the more exacting conditions of the cold room the superior "behavioural energy" of the hybrid mice was not evident (although the hybrids are much more fertile than the inbreds in the cold).

It is suggested that the gnawing of food cubes and the pulling down of the paper strips are analogous to, or aspects of, exploratory behaviour. The latter is evoked especially by novel

stimuli. It seems that the F_1 and the outbred mice have a lower threshold of "arousal" than inbred mice. It is possible that the consequent superior vigour or "nervous energy" has survival value; and that the greater responsiveness, shown by heterozygotic mice, is a regular concomitant of true heterosis (that is, superior fitness of heterozygotes), at least in mammals.

SOME EXPERIMENTS ON CONFLICT MOVEMENTS IN CANADA GEESE. By N. B. G. JONES, *Oxford.*

THE EFFECT OF BENACTYZINE ON CERTAIN BEHAVIOUR PATTERNS OF THE THREE-SPINED STICKLEBACK. By J. M. CULLEN, *Oxford.*

An attempt is being made to investigate the organisation of certain behaviour in three-spined sticklebacks *Gasterosteus aculeatus* using drugs. The aim has been to study the effects of different kinds of behaviour in a variety of situations, including those where a conflict seems to be present. A start has been made with schooling, feeding, territorial activity and courtship. Benactyzine has been chosen because in certain mammals its effects were reported to be relatively specific to certain behaviour systems. The substance is injected intraperitoneally in varying concentrations (100, 20 and 4 mg/Kg. body-weight) and compared with saline controls. Observations are made before, and 2, 4 and 6

hours after injection, and again after 24 hours, by which time the effects of the drug have usually worn off.

Results are available for only a few fish so far, but these suggest that benactyzine depressed courtship activity, as well as the "displacement" fanning and nesting activities normally correlated with courtship. Aggression is also diminished by higher concentrations, while avoidance of a model or a rival is enhanced, so that a male defends a smaller territory. On the other hand, though frightening stimuli normally make a stickleback school more strongly, the effects of benactyzine is to reduce schooling.

EXPERIMENT ON THE EFFECT OF SOCIAL ISOLATION ON REPRODUCTIVE BEHAVIOUR IN THE THREE-SPINED STICKLEBACK. By E. CULLEN, *Oxford.*

A male three-spined stickleback reacts differently to males and females entering his territory. Towards motionless models of males and females the discrimination is less sharp, but there is a clear difference, more attacks being delivered on the male than the female, more courtship towards the female than the male.

This discrimination is present from early in the first breeding season and experiments were carried out to see whether experience of the red-coloured father, who normally rears the young for some days after hatching, might affect it. Three groups of young were reared from the egg to sexual maturity:

1. Isolates—individuals isolated from before hatching from any other fish;

2. Orphans—individuals separated from their fathers from before hatching but reared with young of their own family; and

3. Controls—individuals reared by their father in the usual way.

At the first sign of sexual maturity, the fish living in groups (orphans and controls) were isolated individually so that none gained any new experience of mature males and females. When a male came into breeding condition it was provided with a suitable tank, where it soon built a nest and was ready for testing with male and female models.

Most fish courted and attacked both models, just as wild caught males do, and the analysis of the data so far completed shows no trace of any difference in discrimination between the three different groups. It may be added that the form of the motor patterns involved in the fighting, courting and nesting behaviour and their organisation into the more complex sequences of behaviour were also alike in each group.

During the afternoon there were demonstrations by R. G. B. BROWN, S. PEARCE, P. ELLIS and A. PEARCE, J. M. CULLEN and E. CULLEN to illustrate the experiments described in their lectures during the meeting.

PROCEEDINGS OF THE SECTION OF ANIMAL BEHAVIOR AND SOCIOBIOLOGY (ECOLOGICAL SOCIETY OF AMERICA AND THE AMERICAN SOCIETY OF ZOOLOGISTS)

SYMPOSIUM OF BIOLOGICAL ACOUSTICS

Programme co-sponsored by the Section of Animal Behavior & Sociobiology (Ecological Society of America and the American Society of Zoologists) and the International Committee on Biological Acoustics; Chairman, H. W. FRINGS. The following papers were presented, abstracts of which are available in *Anat. Rec.*, **134**(3), 1959. (The report of this meeting was unavoidably omitted from the previous issue of *Animal Behaviour*).

THE INTERNATIONAL COMMITTEE ON BIOLOGICAL ACOUSTICS: A THREE-YEAR REPORT. By H. W. FRINGS, *Pennsylvania State University*.

REACTIONS OF HONEYBEES TO OSCILLATIONS OF KNOWN FREQUENCY. By H. F. LITTLE, *Pennsylvania State University*.

BASIC PATTERNS OF DIFFERENTIATION IN THE MATING CALLS OF ANURAN AMPHIBIANS. By W. F. BLAIR, *University of Texas*.

VOCALIZATION AS AN ISOLATING MECHANISM IN FROGS. By B. S. MARTOF, *University of Georgia*.

DISTINCTIVE RESPONSES OF TURKEY POULTS TO SPECIFIC SOUNDS. By E. B. HALE, *Pennsylvania State University*.

ACCOUSTICAL COMMUNICATION IN LAYSAN AND BLACK-FOOTED ALBATROSSES. By H. W. FRINGS, and MABEL FRINGS, *Pennsylvania State University*.

UNDERWATER SOUNDS OF THE BERMUDA AREA. By J. M. MOULTON, *Bowdoin College, Maine*.

THE REPRODUCTIVE BEHAVIOR AND SOUND PRODUCTION OF THE SATINFIN SHINER. By J. F. STOUT, *University of Maryland*.

Abstracts of the Proceedings of the Section of Animal Behaviour and Sociobiology (Ecological Society of America and the American Society of Zoologists) of which only title and author are given in *Animal Behaviour* are published in *Anatomical Record* **134**(3), 1959. This also applies to the proceedings that appeared in Volume VII, Pts. 3-4 of *Animal Behaviour*.

Proceedings of the Annual Meeting held at Oklahoma State University, Stillwater, Oklahoma, August 29th-31st, 1960.

Session I. CONTRIBUTED PAPERS
Chairman: R. H. DENNISTON II.

SIZE DISTRIBUTION IN MIXED COLONIES OF ANTS. By R. L. KING and R. M. SALLEE.

SOME ASPECTS OF AGONISTIC BEHAVIOR SEEN IN INITIAL ENCOUNTERS BETWEEN PAIRS OF DEER MICE (*Peromyscus maniculatus rufinus*). By D. F. BALPH.

THE CAUSATION OF AGONISTIC AND SEXUAL BEHAVIOR IN THE CHUKAR PARTRIDGE (*Alectoris graeca*). By A. W. STOKES.

ABSOLUTE AND RELATIVE RATES OF SOCIAL INTERACTIONS IN FLOCKS OF *Gallus domesticus.* **By J. V. CRAIG.**

PIGMENT GENE EFFECTS ON PECK-ORDER BEHAVIOR OF FEMALE TURKEYS. By E. B. HALE and E. G. BUSS.

Session II. CONTRIBUTED PAPERS
Chairman: L. R. ARONSON

SIZE DISCRIMINATION IN THE SIAMESE FIGHTING FISH *(Betta splendens).* **By J. C. BRADDOCK, Z. I. BRADDOCK and G. KOWALK.**

USING LIGHTS TO BYPASS FISH OVER THE McNARY DAM TURBINES. By P. E. FIELDS, G. R. SNYDER and R. W. GREGORY.

HEARING IN SHARKS. I. FREQUENCY RANGE AND THRESHOLDS FOR INTENSITY AND DURATION IN *Carcharhinus leucas.* **By N. KRITZLER and L. WOOD.**

REPRODUCTIVE BEHAVIOR AFTER GONADECTOMY IN MALES OF THE CICHLID FISH *(Aquidens latifrons).* **By L. R. ARONSON, A. SCHARF and K. SILVERMAN.**

COMPARATIVE BEHAVIORAL AND STRUCTURAL EFFECTS OF ANDROGEN INJECTED INTO PREGNANT AND NON-PREGNANT GUINEA PIGS. By M. DIAMOND.

EFFECTS OF PITUITARY GONADOTROPHIC HORMONES ON AGGRESSIVE BEHAVIOR IN MALE STARLINGS. By S. F. MATHEWSON and D. E. DAVIS.

REACTION TIME AT MATING IN RELATION TO GAMETE PRODUCTION IN RABBITS. By E. S. E. HAFEZ.

THE RELATIONSHIP BETWEEN SPATIAL DISTRIBUTION AND WEIGHT OF THE ADRENAL GLANDS POPULATIONS OF PRAIRIE DEERMICE. By C. R. TERMAN.

Session III. CONTRIBUTED PAPERS
Chairman: M. W. SCHEIN

A BEHAVIORISTIC MECHANISM EXHIBITED BY THE WATER SHREW IN SOLVING ITS METABOLIC PROBLEMS. By M. W. SORENSON.

THE EFFECTS OF SINGLE AND REPEATED WHOLE-BODY IRRADIATION WITH FISSION NEUTRONS ON THE VOLITIONAL ACTIVITY OF THE MOUSE. By H. H. VOGEL, Jr. and N. A. FRIGERIO.

MONITORING HEART RATES AND BODY TEMPERATURES OF UNRESTRAINED MAMMALS BY RADIO TRANSMITTER. By W. O. ESSLER and G. E. FOLK, Jr.

THE DEVELOPMENT OF CONTACT BETWEEN MOTHER AND OFFSPRING IN UNGULATES (DOMESTIC SHEEP AND GOATS). By HELEN BLAUVELT, J. B. RICHMOND and U. MOORE.

MODIFICATION OF SEXUAL STIMULI BY IMPRINTING IN TURKEYS. By M. W. SCHEIN.

Session IV. BEHAVIORAL FILMS
Chairman: E. M. BANKS

OKOTOPUS-FORTBEWEGUNG FARBWECHSEL, BAUEN UND PAARUNG. By H. CASPERS, *Zoological Museum, Hamburg.*

BRUTBIOLOGIE DES SCHWARZSPECHTES. By H. SIELMANN, *Zoological Institute of Munich.*

BALZ UND PAARBILDUNG BEI DER STOCKENTE *(Anas platyrhynchos* L.). **By K. LORENZ,** *Max Planck Institut, Buldern Westphalia.*

BIOLOGIE DES HAMSTERS E. PAARUNGS VERHALTEN UND FRÜHE JUGENDSTADIEN. By I. EIBL-EIBESFELDT, *Max Planck Institut, Buldern Westphalia.*

SOCIAL BEHAVIOR IN THE BLACK BUCK. By W. ETKIN, *Yeshiva University, N.Y.*

CONSTITUTIONAL AND ENVIRONMENTAL INTERACTIONS IN REARING OF FOUR BREEDS OF DOGS. PART I. By D. G. FREEDMAN, *Jackson Memorial Laboratory.*

CONSTITUTIONAL AND ENVIRONMENTAL INTERACTIONS IN REARING OF FOUR BREEDS OF DOGS. PART II. By D. G. FREEDMAN, *Jackson Memorial Laboratory.*

THE HOWLER MONKEYS OF BARRO COLORADO ISLAND. By C. R. CARPENTER, *Pennsylvania State University.*

Session V. CONTRIBUTED PAPERS
Chairman: C. C. CARPENTER

PATTERNS OF DISPLAY IN IGUANID LIZARDS. By C. C. CARPENTER.

ON THE SURVIVAL VALUE OF GREGARIOUSNESS IN THE GUPPY *(Lebistes reticulatus).* By P. J. CLARK and W. E. COOPER.

DISPERSAL AND TERRITORIALITY IN BIRDS. By R. F. JOHNSTON.

SEASONAL CHANGES IN ROADSIDE ACTIVITY OF COTTONTAIL RABBITS. By R. D. LORD, Jr.

BEHAVIOR STUDIES OF THE NORTHERN FLICKER. By G. B. HAPP.

BOOK REVIEWS

The Buzzard. By FRANK WENZEL. London: Allen & Unwin. 1959. Illustrated. 35s.

This work consists essentially of a long series of beautiful photographs, supplemented with a short text that has been translated from the Danish. There is no doubting the workmanship of the author, and his factual observations as recorded in the text will presumably be of value to those concerned with this species.

<div align="right">A.N.W.</div>

Dragonflies. By P. S. CORBET, CYNTHIA LONG-FIELD & N. W. MOORE. London: Collins (New Naturalist Series). 1960. Pp. xii + 260. 42s.

This is perhaps the most beautifully-produced of all the New Naturalist series to date. The coloured photographs are delightful and there is a wealth of illustrations of other kinds, including excellent diagrams. All three authors are well-known for their original contributions to the study of dragonflies and two of them have in fact obtained their Ph.D. degrees for theses on this group. That they should have found the time necessary to compile this authoritative, well-documented and readable monograph will place many in their debt, and for present purposes attention should be drawn to the proper place that is afforded to behavioural considerations. Thus, the particular features of copulation in this group are examined and discussed behaviourally, and since fossil evidence for the evolution of the accessory genitalia is lacking, there is ample need for this attempt at reconstruction. Ecologists, too, will appreciate the chapter in which the relationship of dragon-flies to other species is considered.

The appendices provide keys to identification, procedures for preservation of colours and detailed notes on marking.

<div align="right">A.N.W.</div>

Evolution Above the Species Level. By BERNHARD RENSCH, with a foreword by T. DOBZHANSKY. London: Methuen. 1959. Pp. 436. 63s.

This comprises a translation of the 2nd edition of *Neuere Probleme der Abstammungslehre*, published in 1954. The material has therefore been available to interested workers for several years, but it is nevertheless valuable and useful that there should be an English version. It would appear that the translation was in fact complete early in 1956, and to help bridge the gap caused by further delays, there is a postscript of references to several important contributions up to 1958. "In most cases the investigations mentioned fit well in my conception and may be considered as good supplements."

The actual translation appears to have been made by Dr. Altevogt and to be successful, although the text in places remains Germanic in style. The omission of initials in the author index and elsewhere is an allied feature, while the journal abbreviations do not all accord with a recognised mAerican or British systems. These are, however, minor adverse criticisms. Whether or not one agrees with Prof. Dobzhansky's view that the original German work was ". . . one of the great books which has appeared since 1940 and which may fairly be said to have given shape to the modern biological, or synthetic theory of evolution", it is useful to teachers and to those who do not read German easily to have this well-produced translation.

<div align="right">A.N.W.</div>

Animal Behavior. By J. P. SCOTT. University of Chicago Press. 1958. Pp. 300. 37s. 6d.

This is essentially a textbook for students and others who wish to obtain an accurate insight into the scope of animal behaviour studies. It provides and discusses various aspects of animal behaviour with the minimum of specialised terminology, and sometimes with dogmatic explanations of the results of individual experiments. The references are selected so as to provide for "recommended general reading", but "additional references" are provided for all eleven chapters which deal, respectively, with animal and human behaviour; methods of study; anatomy and behaviour; learning; heredity and behaviour; intelligence; social behaviour and social organization; communication; behaviour and the environment; and behaviour and evolution.

Some chapters appear to cover the ground more satisfyingly than others. Not surprisingly, those dealing with heredity and behaviour and with the social aspects of the subject are perhaps the best, and for the particular purposes for which it is intended there will no doubt be widespread usage of this work. The reviewer has found it useful in helping to piece together the conclusions from the variously published dog studies of the author and his fellow workers. Advanced students will require much more detailed

treatment than is possible in such short compass, but should nevertheless profit from reading through this contribution. For general readers there is now a text to replace some of the inaccurate and undocumented books that have not helped the proper dissemination of the subject.

A.N.W.

Annual Review of Psychology. Vol. 11. By P. R. FARNSWORTH & Q. MCNEMAR, Editors. Palo Alto, California: Annual Reviews. 1960. Pp. 553. $7.00 (U.S.A.), $7.50 (elsewhere).

There have been important changes in the planning of this work. Under the new scheme, which is flexible, some topics are to be dealt with annually, some biennially, some triennially and others again at four-yearly intervals or even occasionally as the amount of material demands.

These changes have resulted in the following contributions to the current volume: a review of theory in physiological psychology (K. H. Pribram); behaviour genetics (J. L. Fuller); engineering psychology (A. W. Melton & G. E. Briggs); colour vision (L. M. Hurrich & D. Jameson); perceptual learning (J. Drever); psychological aspects of ageing (J. E. Birren); statistics (L. S. Kogan); individual differences (P. H. Du Bois); personality dynamics (J. W. Atkinson); psycholinguistics (H. Rubenstein & M. Aborn); industrial psychology (B. von H. Gilmer); abnormalities of behaviour (A. Hoffer); psychotherapy (J. B. Rotter); psychopharmacology (S. Rosee & J. O. Cole); developmental psychology (P. Mussen); and social psychology (H. W. Riecken).

Psycholinguistics (presumably representing a section now to be reviewed triennially) has not been dealt with specifically since 1954, and it is both amusing and surprising, in view of the space now devoted to language and hierarchy, that there is no mention of the work of Alan Ross and his popularisers. The chapter on perceptual learning will probably be of greatest interest to readers of this journal and is handled competently and with interesting presentation.

A.N.W.

The Veterinary Annual. By W. A. POOL, Editor. Bristol: John Wright & Sons. 1959. Pp. 388. 42s.

The spate of new veterinary publications includes the first issue of *The Veterinary Annual*, to which there are 43 contributors from ten different countries, some of them eminent and almost all active in their respective fields. A vast range of topics is reviewed, ranging from general articles on trends in different countries or in specialized types of practice to the murine leukaemias and to radiation hazards.

The net result is convincing, but must clearly have involved an almost herculean editorial task that it will surely be difficult to fulfil year by year. It would be invidious to single out individual contributions for consideration: the main impression is one of remarkable uniformity in that such a readable series has been produced, which points again to extreme editorial care.

The reviewer has found the work of decided practical use and has not detected any obvious omissions or errors in those parts where he is familiar with the relevant literature; he would like to wish the new publication every success.

A.N.W.

An Introduction to the Anaesthesia of Laboratory Animals. By PHYLLIS G. CROFT. London: Universities Federation for Animal Welfare. 1960. Pp. 32. 3s. 6d.

This small work is essentially practical and is packed with useful tips, some of them illustrated in a clear and helpful manner. The anaesthetisation of laboratory animals for experimental purposes is, in the U.K., restricted to those persons holding a Home Office licence, with the appropriate certificate, as is indeed made clear in the preface. In these circumstances it is to be hoped that such persons will not be affronted by the inclusion in the glossary (which otherwise incorporates trade names of the common barbiturate anaesthetics and a conversion table) of definitions of scientific terms. Presumably those are intended for others who may use the book, either here or in countries where the regulations are less stringent.

There are clear directions on such points as the choice and use of syringes and needles. Particular attention is paid to the sharpness of the needle and the means of testing it. Local and inhalation anaesthesia are dealt with, as well as that by intravenous or intraperitoneal injection, and there are few practical items that have not been covered. (One, however, would seem to be the induction of vasodilatation, which is not referred to except in relation to the beneficial action of alcohol or acetone used primarily for cleaning the site. Most investigators find the use of materials such as xylol, or mechanical rubbing or—best of all—warmth to be most helpful. Indeed, with such aids the intravenous injection of mice—which on p. 16 is said to require

"considerable skill, practice and patience"—becomes far less formidable, and may readily be learned.) Specific data are given for the rabbit, guinea-pig, hamster, rat and mouse. It is to be hoped that this little book will enjoy the wide-spread popularity it deserves.

A.N.W.

Neuro-Psychopharmacology: Proceedings of the First International Congress of Neuro-Psycho-pharmacology (Rome, 1958). Editors, P. B. BRADLEY, P. DENNIKER & C. RADOUCO-THOMAS. Amsterdam: Elsevier. 1959. Pp. 742. 135s.

The Congress of which this volume constitutes the proceedings was held in Rome in September, 1958, and was attended by more than 500 delegates from 26 countries. Some who attended have since stated that they had some difficulty in following the papers, and they, as well as others, will welcome the publication of this work, which has been achieved remarkably quickly in view of the late decision to undertake it. "The meeting proved to be such a success that it was decided to publish the proceedings and, on the last day, the Executive Committee appointed three of its members to undertake the task, although at this time the delegates were beginning to disperse and no publisher had been approached." The publishers appear to have earned the thanks expressed to them in the preface, for the book has every appearance of careful preparation and a very high standard of production.

Part I (pp. 11-267) deals with four symposium meetings and three plenary sessions, and includes—in all but one instance a record of the ensuing discussions. Part II (pp. 371-717) comprises the texts of original papers—46 dealing with studies on experimental animals, 13 with investigations on normal human subjects and 73 with clinical and therapeutic reports. It is impracticable to attempt to review this considerable array, much of which is, however, of considerable interest to behaviourists generally, and serves to indicate one new field in which behavioural studies are of considerably more than academic importance.

The first symposium, "Methods and analysis of drug-induced behaviour in animals" contains papers by P. B. Bradley and J. Olds, with contributions to the discussion by J. V. Brady, P. B. Dews, P. Glees, B. R. Kaada, D. P. Purpura, C. Radouco-Thomas, L. Weiskrantz, and J. C. Eccles. Bradley emphasizes the caution

necessary in considering possible relationships between the results of animal behaviour experiments with drugs and the effects of the same drugs in man, and considers that confusion may have arisen through too free use of clinical terms to describe behavioural patterns in experimental animals. He proposes that the term psychopharmacology be restricted to human studies, and suggests that behavioural pharmacology would be more appropriate to animal experiments. He draws attention to the artificiality of certain test procedures but, in discussing this point, L. Weiskrantz emphasizes the danger of rejecting procedures simply because the experimental situations involve conditions rather different from those of the so-called natural environment, pointing out that advances often came from abstracting from a complex set of variables those which one feels to be uniquely related to each other. J. Olds discusses results from a technique involving chronic implantation of electrodes at discrete points in the brains of rats with the circuit so arranged that the animal may activate the stimulator by stepping on a pedal. The motivation effect is measured by counting the frequency of this self-stimulation response. The effect of various drugs upon the animal's behaviour would appear to have yielded most valuable information, the materials so tested including chlorpromazine, reserpine, meprobromate, pentobarbital, morphine and amphetamine.

In the sections dealing with clinical applications, one cannot but be surprised by the heroic doses that are sometimes administered. This is true particularly of some of the mono-amine oxidase inhibitors on which the reviewer has had the opportunity of making toxicological studies. It should be pointed out, however, that the acute and sub-acute toxicity of these materials is probably of a somewhat lower order in man than, for example, the dog that is widely used in preclinical testing.

The wealth of material in this volume, mixed though it undoubtedly is, will be of interest and value to a wide range of investigators and clinicians.

A.N.W.

Verhaltensstudien an Katzen. By PAUL LEY-HAUSEN (with English Summary). Berlin and Hamburg: Paul Parey. 2nd ed. 1960. Pp. 128. 18.80 DM.

The second edition of this monograph is in fact a reprint of the first, published in 1955.

Since, through loss of the original review copy in transit to us, we did not review the first edition, it may however be in order to discuss the merits of the work briefly.

This would appear to be undoubtedly the most valuable single contribution to the study of cat behaviour yet published. It is based upon a study of 30 caged and many other free-ranging domestic cats, together with limited obesrvations on captive specimens of other members of the cat family.

The emphases are upon predation—which is described and illustrated in detail—and upon social behaviour. Territorial behaviour relates not only to the home but also to a fairly large hunting territory which—although it may partly overlap the territory of neighbours—is also guarded against trespassers. Agonistic behaviour is described in detail, and it is concluded that a true submissive attitude does not occur in adult cats.

The behaviour of cats in a group confined to a room is interesting. Where there is sufficient space, rigid social order is not established, and although one or two despots may emerge, these appear to enjoy limited prerogatives only. Similarly, one or two "pariah" animals may develop, but generally speaking the outcome of encounters cannot be predicted from the rank of the animals concerned. When the animals are crowded, full intensity of either attack or defence behaviour is rare. Low-ranking animals are forced to be much more demonstrative than those of high rank in order to attain the same social results.

Mating and parental behaviour are described, and special attention is paid to the bite into the nape of the neck, employed during predation, fighting (males only), copulation (males only) and carrying of the young (females only). Disturbance of the balance between the jaw muscles may result in damage or death to the female or young. In the Pantherinae, the copulatory bite has been reduced to an accessory expression of orgasm.

A.N.W.

ANIMAL PRODUCTION
JOURNAL OF THE BRITISH SOCIETY OF ANIMAL PRODUCTION
Contents of Volume 2, Part 2, September, 1960

CRICHTON, AITKEN & BOYNE. The effect of plane of nutrition during rearing on growth, production, reproduction and health of dairy cattle. III. Milk, production during the first three lactations with to first calving.

HOLMES & OSMAN. The feed intake of grazing cattle. I. Feed intake of dairy cows on strip and free grazing.

WIENER. Factors influencing average milk yield of herds at two levels of production.

BARBER, BRAUDE, HOSKING & MITCHELL. Studies on liquid skim milk for growing pigs. The effect of its abrupt removal from the diet and of supplementation with copper sulphate or amino acids.

ROBERTSON, O'CONNOR & EDWARDS. Progeny testing dairy bulls at different management levels.

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